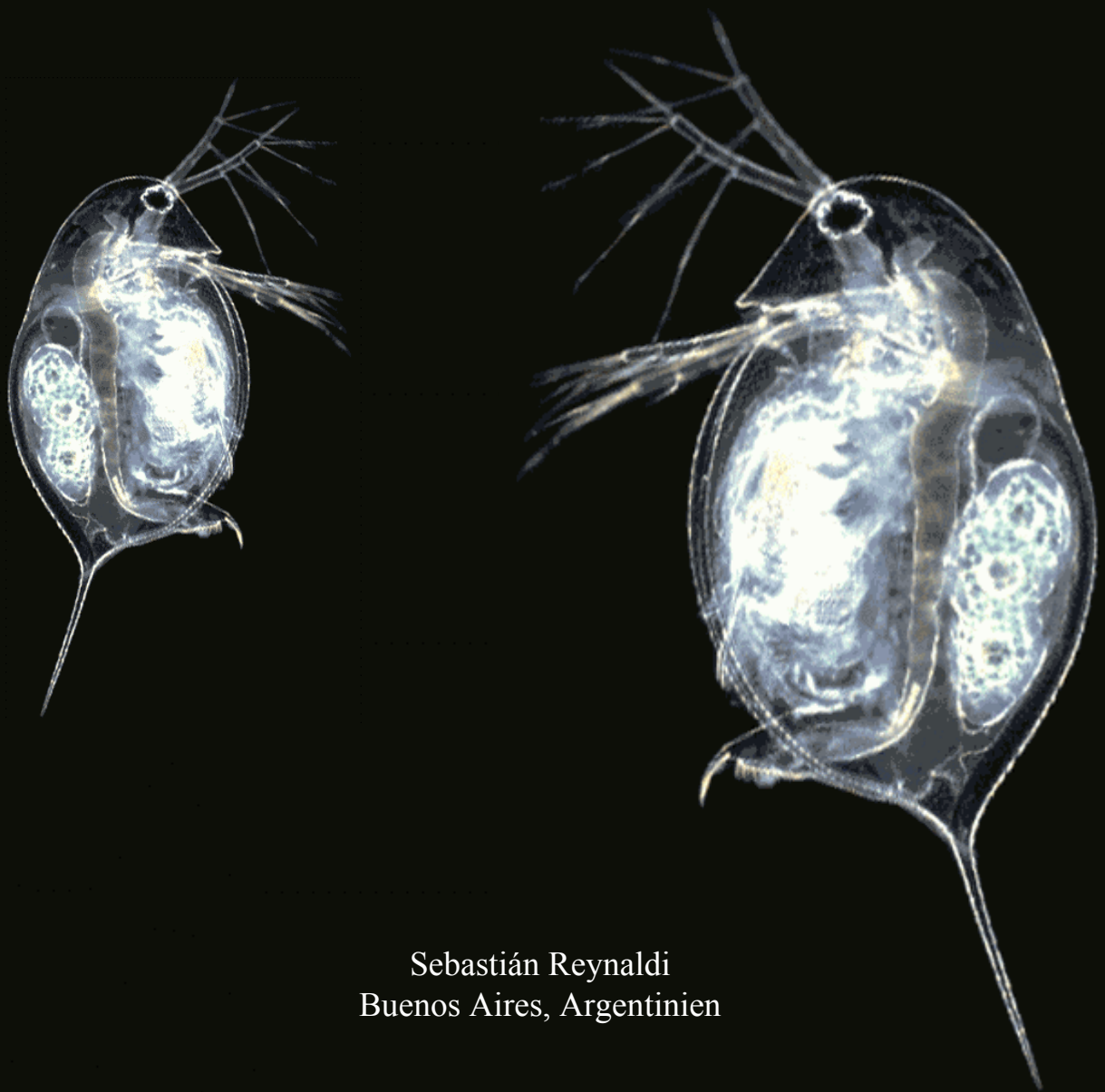
Decreasing uncertainties in pesticide risk assessment: influence of exposure duration on population-level responses of *Daphnia magna S*traus to the pyrethroid fenvalerate



Sebastián Reynaldi
Buenos Aires, Argentinien

Decreasing uncertainties in pesticide risk assessment: influence of exposure duration on population-level responses of *Daphnia magna S*traus to the pyrethroid fenvalerate

Von der Gemeinsamen Naturwissenschaftlichen Fakultät
der Technischen Universität Carolo-Wilhelmina
zu Braunschweig

zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr.rer.nat.)

genehmigte

DISSERTATION

von Sebastián Reynaldi
aus Buenos Aires, Argentinien

1. Referent: Prof. Dr. G. Rüppell
2. Referent: PD Dr. Liess

eingereicht am: 11. Aug. 2004
mündliche Prüfung am: 15. Nov. 2004

Anlage 4
Muster zu § 4 Abs. 85) für Dissertationen

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Gemeinsamen Naturwissenschaftlichen Fakultät, vertreten durch den Mentor und den Betreuer der Arbeit in folgenden Beiträgen vorab veröffentlich:

Publikationen

Reynaldi S, Liess M. Response and Recovery of *Daphnia magna* Straus to the Insecticide Fenvalerate: Relevance of Exposure Duration. (acceptable with correction).

Reynaldi S, Jung K. and M. Liess. Feeding responses: a link between individual to population levels responses of *Daphnia magna* Straus following short-term (24-h) exposure to the pyrethroid fenvalerate. (in preparation).

Reynaldi S, Liess M. Varying exposure duration under low-food conditions: Its influence on responses of *Daphnia magna* Straus at individual-level and their extrapolation to population-level. (in preparation).

Tagungsbeiträge

Nov 2003 Reynaldi, S, M. Liess and K. Jung. Use of ^{15}N tracer technique to link short-term exposures of fenvalerate and chronic responses of Daphnia magna Straus: beyond Just counting dead animals. SETAC Latin America 6th Annual meeting, Buenos Aires, Argentina.

Nov 2003 Reynaldi, S, and M. Liess. Efectos crónicos y recuperación en Daphnia magna Straus expuesta por 24hs al piretroide fenvalerato. SETAC Latin America 6th Annual meeting, Buenos Aires, Argentina.

Nov 2003 Reynaldi, S, and M. Liess. Influencia de la limitación recursos en las respuestas demográficas de Daphnia magna Straus expuesta al piretroide fenvalerato en forma continua. SETAC Latin America 6th Annual meeting, Buenos Aires, Argentina.

Nov 2003 Reynaldi, S, and M. Liess. Efectos letales posteriores a una breve exposición (24h) al insecticida piretroide fenvalerato en neonatos y adultos de *Daphnia magna* Straus. SETAC Latin America 6th Annual meeting, Buenos Aires, Argentina.

Nov 2003 Reynaldi, S, and M. Liess. Influencia de la disponibilidad de recursos alimenticios y duración de la exposición en la relación concentración respuesta de *Daphnia magna Straus* al insecticida piretroide fenvalerato. SETAC Latin America 6th Annual meeting, Buenos Aires, Argentina.

Oct 2003 Reynaldi. S, Liess M. and Klaus Jung. Application of ^{15}N tracer technique for the determination of fenvalerate effects in *Daphnia magna* Straus German chemical association (GDCh), Annual meeting 2003. October 6-11, München, Germany.

Apr 2003 Pieters B., Reynaldi S, and M. Liess. Effects of food limitation on the sensitivity of Daphnia magna to fenvalerate. SETAC Europe 13th Annual meeting, Hamburg, Germany.

Apr 2003 Reynaldi, S, Wilson S. , Pieters B., Duquesne S. and M. Liess . Influence of the exposure duration on the responses of *D. magna* Straus to the pyrethroid insecticide fenvalerate: Pulse vs. continuous exposure. SETAC Europe 13th Annual meeting, Hamburg, Germany

Oct 2002 Reynaldi S, Duquesne S and .M. Liess. Neonates fitness: A link between individual and population level. Risk assessment section of annual meeting German chemical association (GDCh),. Braunschweig, Germany

Apr 2002 Reynaldi S., Duquesne S. and .M. Liess. Neonates fitness: A link between individual and population level in ecotoxicological assessment risk at SETAC Europe 12th annual Vienna, Austria.

Dedicated to my wife, Lali Barrera, my brother-in-law, Nicolás Lucero, and my friend, Andrea Müller for their collaboration

Abstract

This study investigated chronic effects of the pyrethroid insecticide fenvalerate on *Daphnia magna* Straus. Four treatments combining exposures to continuous (21d) - and -pulse (24h) contamination, and normal- and low-food conditions, were tested using a chronic test derived from the 21d-standard-reproduction test. Additionally, the ingestion activity was investigated, applying the ^{15}N-tracer incorporation and filtration rate measurements. Fenvalerate affected the survival and delayed the maturity. Mortality increased with increasing exposure duration and decreasing food concentration. The maturity was more severely delayed with increasing exposure duration, but not with decreasing food. The effects on survival and maturity reduced the population growth rate. No substantial recovery occurred in the continuous-contamination treatments, where mortality explained more than 98% of the population growth rate ($p< 0.01$). In contrast, the population growth rate recovered in the pulse-contamination treatments. However, it was in minor extent under low-food conditions. The investigation of ingestion activity revealed that the pulse-contamination resulted in a transient reduction of the filtration rate, which retarded somatic growth and thus delayed maturity. The delay in the maturity of exposed individuals resulted in a severe inhibition in the number of neonates per living female. However, this initial inhibition was rapidly attenuated as the females progressed with the reproduction. The degree of attenuation depended on the total number of neonates at the end of the experiment, which is dependent on the food conditions. For this reason, the recovery of the population growth rate was more substantial under normal-food conditions. These results showed that fenvalerate toxicity decreased with pulse-contamination and increased with low-food conditions. Since these conditions may occur in the field, future risk assessments of pesticide should consider food conditions and exposure duration.

Abstrakt

In dieser Arbeit wurden chronische Effekte von Fenvarelate, einem Insektizid auf Pyrethroidbasis, auf *Daphnia mangna* Straus untersucht. Vier verschiedene Ansätze, in denen zum einen die Expositionsdauer (kontinuierliche Exposition (21d) und Kurz-Zeit-Exposition (24h)) und zum anderen das Nahrungsangebot (normal und Nahrungsmangel) kombiniert wurden, wurden im Standard-Reproduktionstest (21d) untersucht. Zusätzlich wurde die Nahrungsaufnahmeaktivität unter Anwendung von ^{15}N als Tracer und der Messung der Filtrationsrate analysiert.

Fenvarelate beeinflusste die Überlebensrate und verzögerte die Geschlechtsreife von *Daphnia mangna.* Die Sterblichkeitsrate stieg sowohl mit steigender Expositionsdauer und als auch mit verringerter Futterkonzentration an. Mit steigender Expositionsdauer wurde ebenfalls die Geschlechtsreife verzögert, Nahrungsmangel allein zeigte jedoch keinen Effekt darauf. Die Beeinträchtigungen der Überlebensrate und der Geschlechtsreife führten zu einer stetigen Reduktion der Populationswachstumsrate.

Bei den Versuchen mit kontinuierlicher Exposition wurde die Populationswachstumsrate zu 98% durch die Sterberate bestimmt, es trat keine Erholung auf. Im Gegensatz dazu konnte unter Kurz-Zeit-Exposition ein Erholungseffekt der Population beobachtet werden. Dieser trat auch unter Nahrungsmangel auf, wenn auch in einem geringeren Ausmaß.

Die Untersuchungen der Kapazität der Nahrungsaufnahme zeigten, dass eine Kurz-Zeit Exposition eine vorübergehende Reduktion der Filtrationsrate auslöste, was eine Hemmung des Wachstums der Tiere und eine Verzögerung der Geschlechtsreife verursachte. Diese Verzögerung führte zu einer deutlichen Verringerung der Anzahl der Nachkommen pro Weibchen. Jedoch wurde diese anfängliche Hemmung schnell mit der beginnenden Reproduktion abgeschwächt. Dieser Grad der Abschwächung war abhängig von der Gesamtzahl der Nachkommen zum Ende des Experiments, was wiederum mit dem Nahrungsangebot zusammenhing. Aus diesem Grund war trat unter Bedingungen mit normalem Nahrungsangebot ein deutlicherer Erholungseffekt zu beobachten.

Diese Ergebnisse zeigen, dass Nahrungsmangel die Toxizität von Fenvarelate auf die Population von *Daphnia magna* verstärken kann, wogegen eine Kurz-Zeit Exposition zu einer Verringerung führt. Künftige Untersuchungen zur Risikoabschätzung von Pestiziden sollten daher das Nahrungsangebot und die Expositionsdauer berücksichtigen, da Nahrungsmangelsituationen und Kurz-Zeit Expositionen eher realistischen Feldbedingungen entsprechen.

INDEX

CHAPTER I

GENERAL INTRODUCTION

1.1- Pesticide risk assessment, tiered-process proposal.

A tiered or phased approach has been recommended as a rational procedure to assess the risks of toxicants or other stressors by many authors and regulatory authorities (Urban and Cook, 1986; European Union 1991; Suter 1993; SETAC 1994; OECD 1995; Environment Canada 1997; ASTM 1997; EPA 1998). The purpose of a tiered process is to provide a logical progression of tests and risk assessment approaches to address the potential risks of toxicants to aquatic ecosystems. The common feature of all tiered regulatory processes is a progression beginning with conservative assumptions and moving toward more realistic estimates. Tiered processes tend to be cost-effective ensuring that resources are expended on pesticide products/issues meriting attention. For pesticides, the ECOFRAM Aquatic Workgroups[1] propose a process consisting of four tiers, each structured similarly, with a Problem Formulation phase, Analysis phase, and Risk Characterization phase (Figures 1, 2 and 3). Tiers are differentiated primarily by the data likely to be available at that stage in the risk assessment process and the relative cost of achieving risk refinement appropriate for that tier of analysis. The tier process amounts to a cost-benefit balance in which additional resources are expended with progressive tiers to reduce uncertainty and address variability in risk assessment and characterization. While a tiered approach provides necessary structure and organization, defines a progression for refined assessments, and allows regulatory decision points, the separation between the tiers is not intended to be rigid. It is recognized that to the extent possible, all relevant data should be utilized and that valid effects and exposure comparisons may cross tier boundaries. For example, higher tiered effect data, if available, may be compared with exposure estimates generated at lower tiers (or vice versa).

[1] Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) and FIRA refers to the Federal Insecticide, Fungicide, and Rodenticide Acts of U.S.

1.1.1-Screening, Tier 1

The objectives of Tier 1 are to identify those pesticides, which a risk assessment indicates with high confidence to have minimal environmental/ecological concerns (e.g. minimal aquatic ecological risks). Tier 1 prioritises worst potential environmental exposures, and finds out whether acute or chronic concentrations may be of concern for sensitive taxa (e.g. invertebrates, fish or aquatic plants).

Tier 1 generates a simple deterministic risk quotient based on the standard battery of toxicity data and a simple and conservative edge-of-field scenario. The exposure scenario is selected to represent a defined exposure "severity" set by regulators such that a Tier 1 indication of "no risk" is protective at the defined level of concern. The output of Tier 1 provides a decision with which the risk assessor is confident that there is minimal aquatic ecological concern associated with the product/use pattern, or the indication that progression to Tier 2 is essential.

1.1.2-Basic risk characterization, Tier 2

The objectives of Tier 2 are to provide probabilistic expressions for potential risk associated with use patterns/taxa combinations identified in Tier 1, confirm that risk predicted in Tier 1 still applies when physical-chemical processes and environmental fate parameters are better represented. In addition, it intends to provide an estimate of the variation of risk temporally, regionally and seasonally across a wide range of conditions of pesticide use, and permit preliminary evaluation of basic mitigation and management options, if there is sufficient understanding of the ecological risk. Tier 2 provides a probabilistic assessment of potential risk using complete concentration-response relationships derived from the standard battery of toxicity test data from Tier 1 combined with a multi-regional exposure assessment that provides the distribution of concentration in surface water adjacent to treated fields. At Tier 2, the environmental fate behavior data generated as part of the standard battery of laboratory and field studies is incorporated into the exposure assessment modeling. Finally, Tier 2 needs to provide guidance on which Tier 3 options to consider. Currently, in many cases, compounds entering Tier 2 also require some form of Tier 3 assessment. The assessment step (diamond) identified as "Evaluate Risk Characterization and Explore Options" in the Tier 2 section of Figure 1 represents a process common to Tiers 2, 3, and 4. It is a multi-step process for the risk assessor to

decide whether the uncertainty around the risk characterization is sufficiently well understood to permit any further evaluation and, if so, whether the process then involves a more detailed exploration of the output.

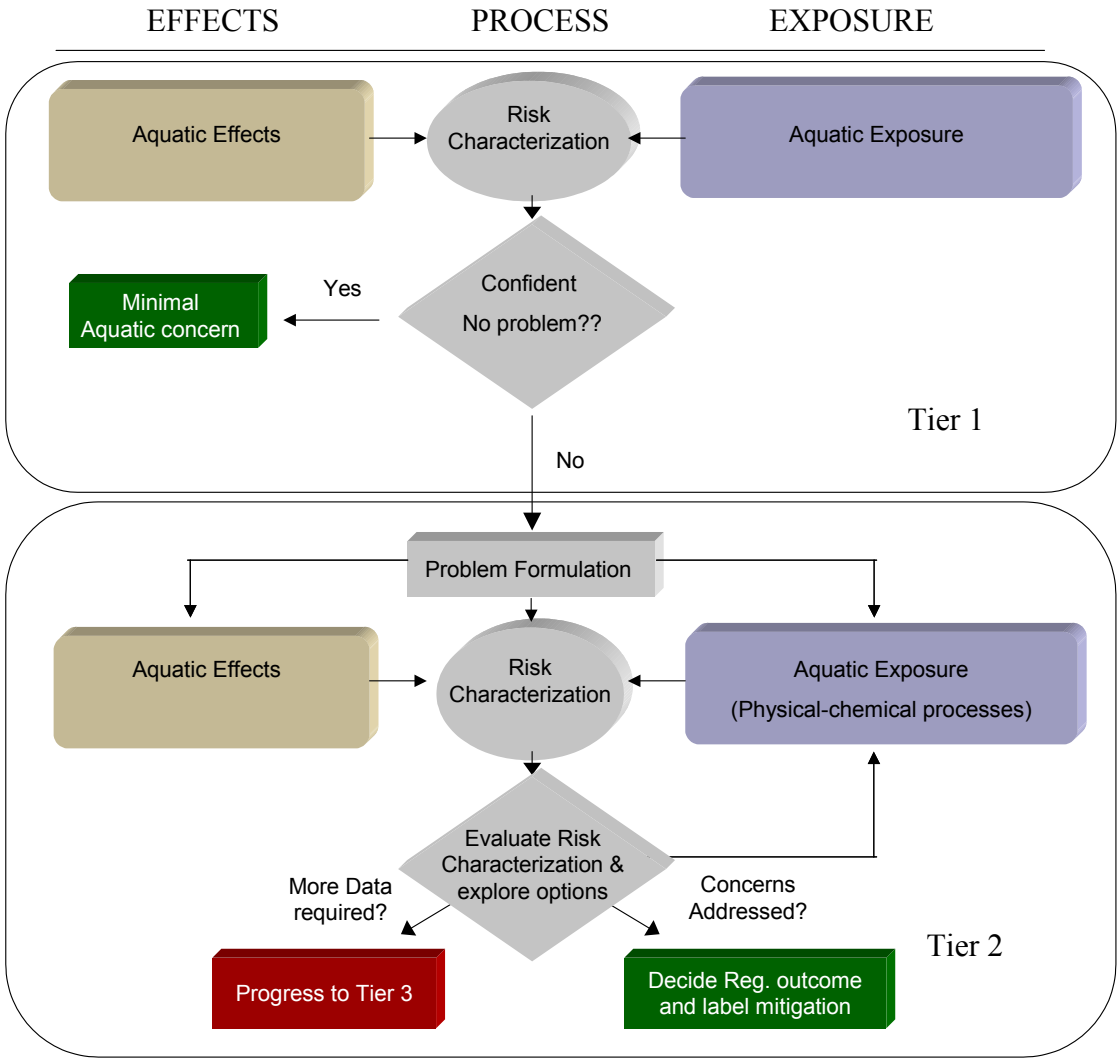


Fig. 1. ECOFRAM Risk Assessment Process - Tier 1 Screening and Tier 2 (and basic risk characterization).

1.1.3- Refining estimates of risk and uncertainty, Tier 3.

The objective of Tier 3 (Fig. 2) is to provide a probabilistic assessment of potential risk using similar approaches to Tier 2, but refined by acute and chronic toxicity studies. It should include:

1. Additional species.
2. Investigations of the toxicity associated with exposure duration
3. Chronic toxicity studies to extrapolate effects from individual-level to population level.
4. Sediment toxicity.
5. Additional laboratory or pseudo field environmental fate studies.

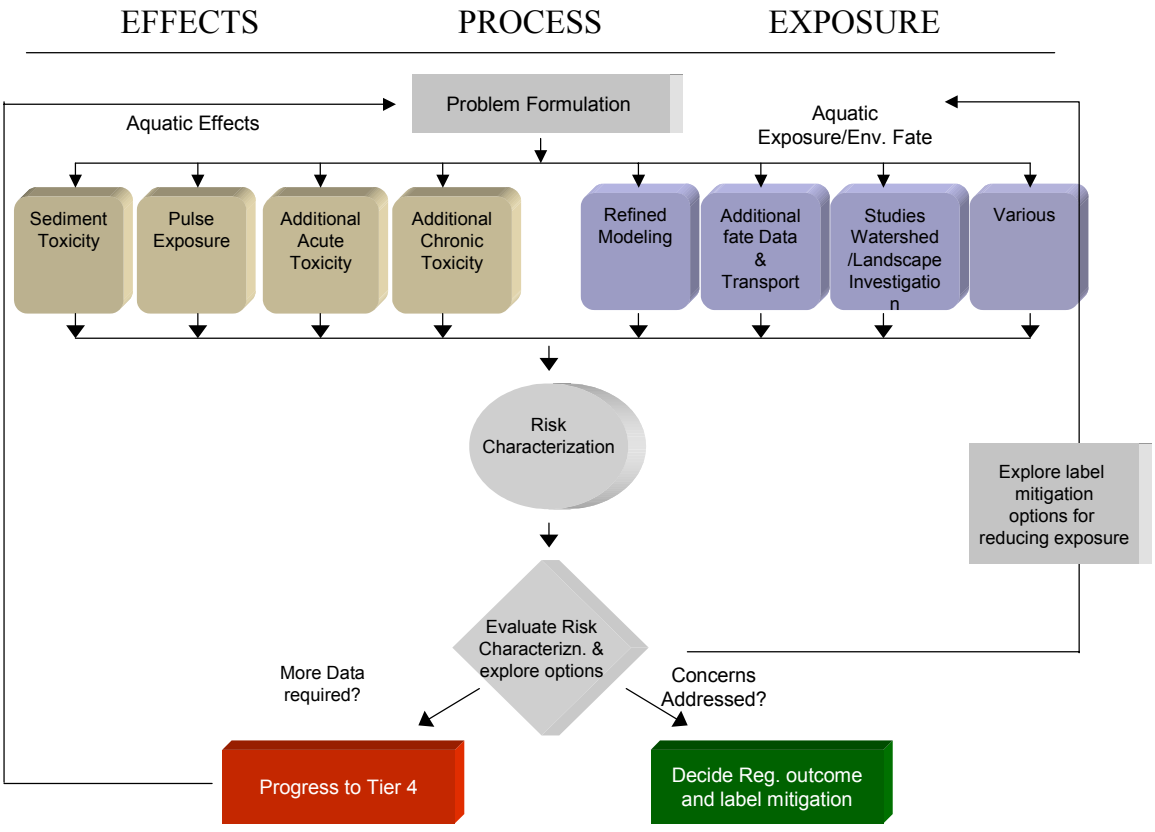


Fig. 2. Tier 3 - "Toolbox" approach, refining estimates of risk and uncertainty.

It is proposed that the selection of one or more of the Tier 3 options should be based on expert judgment. The concept to consider is one of identifying the most appropriate tool or tools from a well-equipped "toolbox." Assuming clear generic guidance on the tier system is defined, it is likely that much of the work encompassed by Tiers 2 to three can be conducted by the registrant prior to discussion with regulators. Nevertheless, discussion between risk managers

and risk assessors may often be helpful to share information on likely issues associated with a product/use pattern and to benefit from expert Agency opinion at any point in this risk assessment process.

1.1.4- Major programs, sophisticated modeling or mitigation validation studies, Tier 4.

Tier 4 (Fig. 3) generally involves broad reaching experimental or monitoring programs designed to definitely characterize key aspects of the toxicity or exposure profiles. Tier 4 programs include widespread monitoring, detailed investigation of the efficacy of mitigation, and highly refined watershed evaluations and modeling. In addition, the Tier 4 can include benchmark modeling relative to existing chemical data, modeling of population or ecosystem dynamics, and microcosm or mesocosm studies.

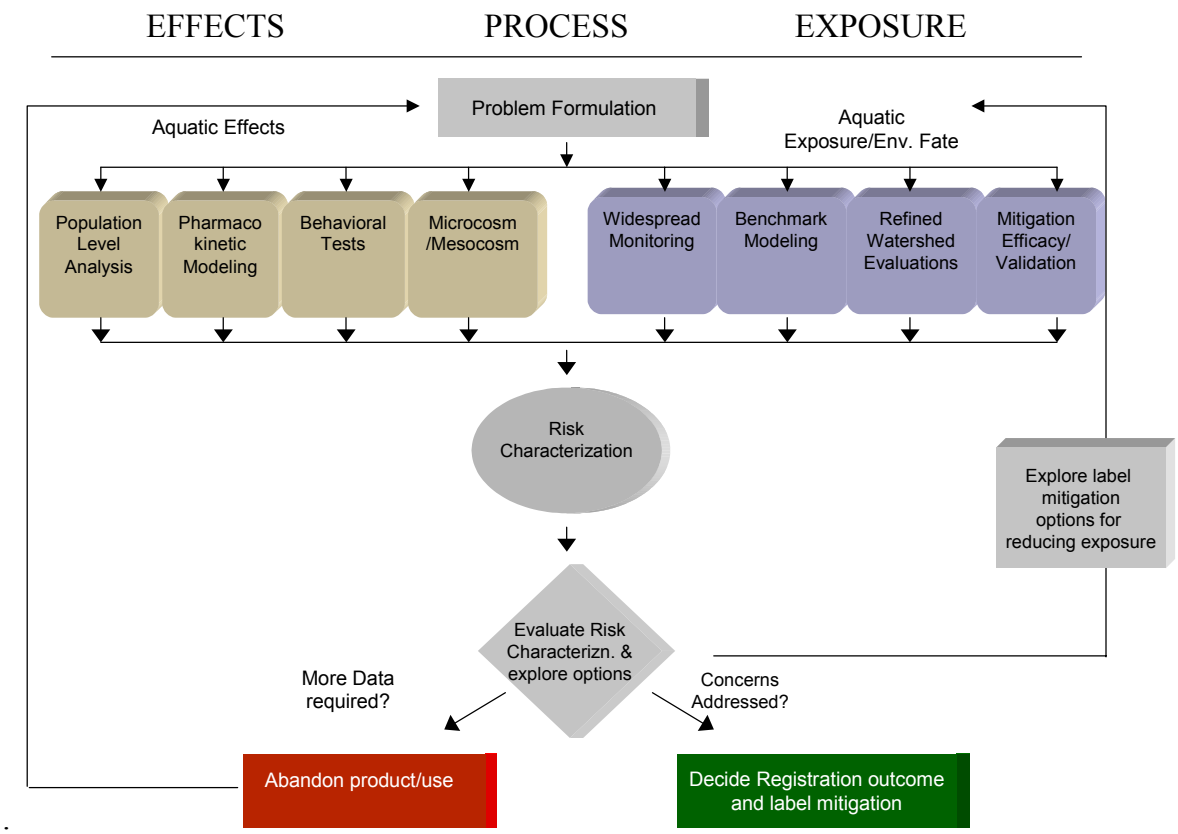


Fig. 3. Tier 4 - Major programs, sophisticated modeling or model validation studies.

Which options are selected at Tier 4 depends entirely on the risk assessment issues that remained after Tier 3. Both Tiers 3 and 4 are intended to be highly flexible. Consultation between registrants and regulators is essential at this stage because of the extraordinary cost associated with the programs. This tier will be decisive about the use of the pesticide.

1.2- Description of the problem

From proposal resumed above, many questions raised and issues emerged for discussion and further development. The ECOFRAM Aquatic Workgroups[1], who formalized the proposal, gave priority to a list of gaps of knowledge that, among others points, includes:

1- The effects of pulse or short-time exposures to pesticides.
2- The extrapolation of toxic effects from individual to population-level.
3- Feeding responses: link between individual and population levels.

These points will be introduced the next sections of the introduction, and experimentally investigations related to them are summarized in followed chapters.

1.2.1- Risk environmental assessment of pulse or short-time exposures to pesticides

Ideally, representative species would be tested by exposure to the chemical of concern under natural (field) conditions over a period similar to that expected under realistic conditions. Generally, this is not feasible because keeping track of a group of individuals in the field is difficult. In addition, continual measurement of field exposures to wild organisms can be costly and problematic. Instead, toxicity tests are generally performed in the laboratory by exposing individuals to a tested chemical continuously. This kind of testing, under controlled conditions, provides the best opportunity to determine the effects of chemical on the representative species (i.e. *Daphnia magna*) under the worst circumstances. However, these circumstances may not be common in the field, and the effects of pulsed exposures represents a source of uncertainty in the pesticide risk assessment.

Contamination of surface waters from pesticides typically occurs in single or repeated pulses due to agricultural runoff, spray drift, or intermittent urban and domestic use. These input

[1] Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) and FIRA refers to the Federal Insecticide, Fungicide, and Rodenticide Acts of U.S.

patterns typically result in a period of high concentration followed by a decline in concentration due to hydrological dilution, degradation, or partitioning from water to air or sediments. Standard laboratory toxicity tests using constant exposure concentrations typically do not investigate the toxicity of pulse exposures (Hosmer et al., 1998). Evaluation of acute and chronic effects in aquatic environments due to pulsed exposures has been addressed by numerous authors (Jarvinen et al. 1989; Holdway et al., 1994; Hosmer 1996; Naddy et al, 2000). These authors lighted that episodic pollution events may not adequately be addressed by conventional toxicity testing methods with fixed duration continuous exposure.

In addition, once a compound has entered the aquatic environment its distribution and hence potential to cause adverse effects will depend on a number of factors including the potential for abiotic (e.g. hydrolysis, oxidation, photolysis) and biotic (aerobic and anaerobic) degradation and the partitioning behaviour of the compound to organic matter. The last is especially relevant for hydrophobic pesticides such as pyrethroids.

1.2.1.2-Dissipation processes

Concentrations of hydrophobic pesticides in streams decrease quickly because of adsorption processes (Farmer et al., 1996). For example, the introduction of agricultural the pyrethroid insecticide fenvalerate into streams due to runoff, leads to short-term peak concentrations (Schulz and Liess, 1999; Liess et al., 1999). In the natural environment, water contains dissolved and particle organic matter that have been shown to interact with hydrophobic pesticides resulting in a quickly reduction of their bioavailability. Previous studies have demonstrated that the presence of dissolved and particle organic matter reduce accumulation and toxicity of fenvalerate (e.g. Day, 1991; Schulz and Liess, 2001). Only freely dissolved compounds are generally assumed to impaired aquatic organisms (Day, 1991).

Studies using the hydrophobic synthetic pyrethroids, lambda cyhalothrin (log K_{ow} = 6.8), fenvalerate and its stereoisomer esfenvalerate (log K_{ow} = 6.2) have demonstrated that the presence of soil or sediment in the test system results in a significant reduction in the observed effect of a pesticide (Boxall et al, 2001). This can be explained by the fact that pesticide is available in the water column for short time due to partitioning of the compound from the aqueous phase to organic mater in the sediments (Maund et al., 1998). Therefore, to obtain toxicity data relevant to this field situation, effects of short-term exposures must be assessed.

Fig. 4. Agricultural runoff events that can result in pulse exposure to aquatic organisms (60 Ways Farmers Can Protect Surface Water, This Land. Online Publication. College of Agricultural, University of Illinois).

In addition, the most common tests do not consider effects occurring subsequent to the exposure period (Abel, 1980; Cold and Forbes, 2004) and rely largely on standard test species such as *Daphnia magna* (McCahon and Pascoe, 1988). Field populations of aquatic organisms, particularly those inhabiting flowing waters, are likely to be exposed to short pulses of pesticide exposure following periods of spray drift, surface runoff, or drain flow (Krueger, 1998 and Liess et al., 1999). These pulses may extend anywhere from a few minutes to several hours, depending on the properties of the pesticide and the characteristics of the water body. It is thus of interest to examine how the effects of such more realistic exposure scenarios, including effects that might persist following the end of the pulse, compare with effects predicted from standard risk assessment scenarios.

1.2.2- Effects Extrapolation from individual to population-level endpoints

The population is the smallest self-reproducing biological unit that is persistent in time. For this reason, ecological risk assessors have long argued that the abundance and persistence of populations of organisms are more relevant as endpoints for ecological risk assessment than are responses at individual-level (Calow, 1997). External factors such as limitations of food availability or other resources affect individuals, determining birth and death rates. However, the net effect of the external factors acting on individuals can be determined by population growth rate because it is the integral of individual birth and death rates (Sibly at al, 2000).

Forbes & Calow (1999) have found that population-level data (i.e. population growth rate) are superior to other types of toxicity data. Many questions of interest in risk assessment relate to effects on the abundance, production, and persistence of populations. Sources of uncertainty for population-level analysis reside in the lack of understanding of population dynamics. The true nature of how populations are maintained under natural conditions is not fully understood; therefore, the impact of changes to birth rates, death rates, and recruitment caused by the introduction of toxicants is not fully understood (Ratte, 1996).

Population-level parameters such as population growth rate can be calculated for some species tested in the laboratory (e.g., *Daphnia magna*) helping to extrapolate toxicant effects from individuals to population-level. There are two ways of expressing population growth rate: the intrinsic rate of increase (r) and the population multiplication factor (λ), which is the antilogarithm of r. The population growth rate is a measure of the ability of a population to increase logarithmically in an unlimited environment. The calculation of the population growth rate requires knowledge of a population's survivorship and fecundity schedule, which is usually recorded from life table response experiments (LTRE)[2], such as the Daphnia reproduction test. The growth rate is then calculated by iteratively solving Equation 1:

$$1 = \sum l_x m_x e^{-rx} \quad (1)$$

[2] Life table response experiments (LTRE) quantify the contribution of each of the vital rates to the variability in population growth rate due to environmental stress

Where x is the age of the cohort, l_x is the proportion of individuals surviving to age x, m_x is the number of females produced per female of age x, and r is the intrinsic rate of increase for the population. Positive values of r indicate exponential population increase, r equal to zero indicates that the population is stable, and negative values of r indicate that the population is declining exponentially and heading toward extinction.

Matrix algebra is another approach used to generate r or λ (Caswell, 2000). In this case probabilities of survivorship and reproduction are organized into a transition matrix, which is then multiplied by a population vector containing information about the size of the population at each age x. Repeated iterations of the multiplication of the population vector, and the transition matrix result in a stable population vector. Thereby, further multiplication by the transition matrix is equivalent to multiplication of the population vector by a constant. Mathematically, this may be written as:

$$\lambda Z = LZ \quad (2)$$

Where L is the transition matrix, Z is the population vector at equilibrium, and λ is the population growth rate. Once the population vector has reached equilibrium, this equation can be solved for $\lambda (= e^r)$.

The population growth rate explicitly integrates individual-level responses to toxicants at population-level; consequently, it is, by definition, a better ecotoxicological endpoint for population responses than any single individual-level endpoint. Population growth rate is more ecologically relevant than any individual-level traits that are currently measure (e.g. acute and chronic survival, egg production). Studies of effects of insecticides on zooplankton and terrestrial arthropods have shown that lethal concentration (i.e. LC50) values were not a good predictor of effects at population- level (Day and Kaushik 1987; Daniels and Allan 1981; Walthall and Stark 1997).

Day and Kaushik (1987) showed that *Daphnia galeata mendotae* populations exposed to sublethal concentrations of the pyrethroid insecticide fenvalerate were able to sustain a rate of increase similar to that of unexposed controls. Daniels and Allan (1981) showed similar results in *Daphnia pulex* with the insecticide dieldrin. Working on *Acyrthosiphon pisum* and its response to the miticide imidacloprid, Walthall and Stark (1997) showed that the populations exposed to the 72-h LC60 were able to maintain rates of population increase similar to untreated controls. Walthall and Stark (1997) attributed this lack of population level response, even at exposure concentrations above the LC50, to compensatory mechanisms where the unaffected individuals were able to maintain reproduction rates similar to control.

Population growth rate is being used more frequently to evaluate toxicity (Stark and, Walthall, 1997; Kammenga et al., 1996; Forbes and Calow, 1999). Real applications of demographic toxicology typically incorporate life table parameters in meaningful comparative exercises; for instance, life table parameters for unexposed populations and populations exposed to various concentrations of a toxicant/pollutant can be incorporated, and ensuing population responses can be compared (Stark and Banks, 1999). This approach is especially appealing because ecological and toxicological parameters are combined, resulting in better predictions about the effects of toxicants at the population-level.

Although it may never be feasible to predict the consequences of exposure to a specific toxicant for a specific species a priori, relating individual life-history traits to population growth rate through the application of elasticity analyses can provide a powerful tool for interpreting the consequences of individual-level impairments for population (Forbes el al., 1999).

1.2.2.1- Sensitivities and elasticities

Sensitivities are derived from the population model by performing partial differentiation of the characteristic polynomial with respect to each individual life-history traits. Since these life-history traits are measured on different scales, e.g. survival probability is measured on a scale from 0 to 1 and fecundity is measured from 0 to x, sensitivities to different life-history traits can not be compared directly. A general method to overcome this difficulty was proposed by Kroon at al (1986) by introducing the term "elasticity".

$$e_x = \frac{x}{\lambda} \cdot \frac{\partial \lambda}{\partial x} \quad (3)$$

Where x demotes the life-history trait and $\partial\lambda/\partial x$ is the sensitivity of λ ($= e^r$) to changes in x.

Elasticity makes it possible to compare sensitivities of each individual life-history traits on a common scale. Thus, elasticity estimates the proportional sensitivities and the relative contribution of life history traits to the population growth rate. Refinements of the basic methodology have been described by van Groenendael et al. (1994) and van Tienderen (1995). Caswell (1996) used elasticity to predict the influence of changes in survival and reproduction due to hypothetical pesticide on the population growth rate.

1.2.2.2- Two-stages model

The population growth rate also can be calculated using the two-stage model. In this model, individuals are classified only as juveniles and adults.

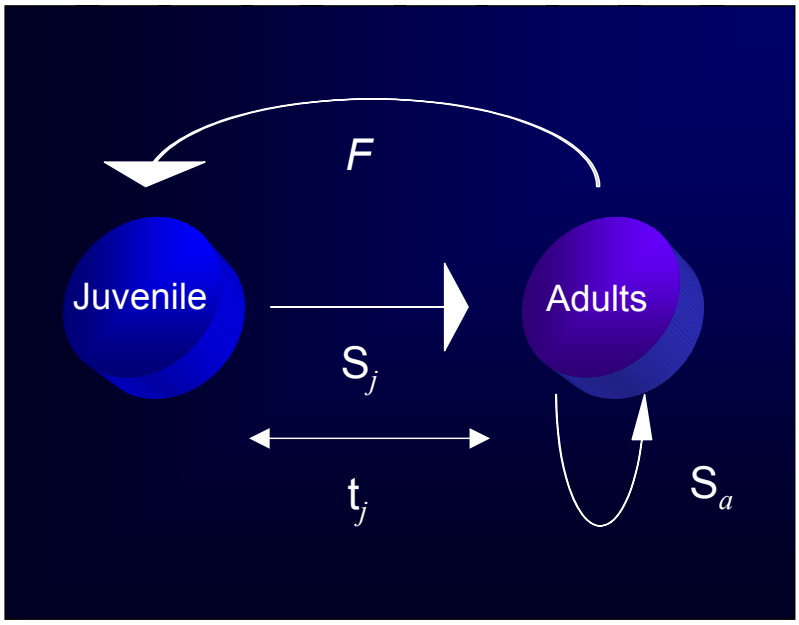


Fig.5. Graphic representation of the two-stage model -juveniles and adults- where *tj* is the age at first reproduction, *Sj* is survivorship until first reproduction, *F* are offspring at each breeding attempt, and *Sa* is female survivorship after first reproduction.

This model was previously analysed by Levin et al. (1996) and Sibly et al. (2000) and, in the present study, was applied to calculate the contribution of the individual life-history traits to the population growth rate and the elasticity of it to them. In the two-stage model, the age at first reproduction is *tj*, survivorship until then is *Sj*, females produced *F*, offspring at each breeding attempt, breeding attempts are t_a apart, and female survivorship between them is *Sa,* then the Euler-Lotka equation (1) can be rewritten:

$$1 = e^{rt_j} S_j n(1 + e^{-rt_a} S_a + e^{-2rt_a} S_a^2 + e^{-art_a} S_a^3 + ...) \quad (4)$$

Writing $e^{-rt_a} S_a = y$, it results in:

$$1 = e^{-rt_j} S_j n(1 + y + y^2 + y^3 + ...)$$

$$= \frac{e^{-rt_j} S_j n}{1 - y} \quad (5)$$

Using a formula of a geometric series, substituting for y and rearranging (Sibly and Calow, 1990), it results in:

$$1 = e^{-rt_j} S_j F + e^{-rt_j} S_a \quad (6)$$

In this way, the Euler-Lotka equation is rewritten in order to calculate the population growth rate of a population divided only in adults and juveniles. This can simplify the collection of information and the posterior analysis about the relation of the individual life-history traits of vital rates and the population growth rate. It is assumed that the probability of survivorship and reproduction are constant for into each stage (Sibly, 2000).

1.2.2.3- Contribution analysis

The contribution of toxicant effects on reproductive output or fecundity (F), age at first reproduction (tj), juvenile survival (Sj) and adult survival (Sa) to the population growth rate can be calculated from the equation (2) proposed by Levin et al. (1996).

$$\lambda^{(k)} \approx \lambda^{(c)} + \frac{\partial \lambda}{\partial F} \Delta F + \frac{\partial \lambda}{\partial t_j} \Delta t_j + \frac{\partial \lambda}{\partial S_j} \Delta S_j + \frac{\partial \lambda}{\partial S_a} S_a$$

(7)

Where ΔF, Δt_j, ΔS_j and ΔS_a are the differences between treatment and control values for each of the parameters, and the sensitivities are evaluated at the mean of the two parameters sets. The same approach is used in order to calculate contributions of each of the four parameters mentioned above.

1.2.3. Feeding responses: link between individual and population levels.

Ecotoxicologists study the fate and effects of toxicants in ecosystems with the aim of understanding how toxicants affect the structure and functioning of populations, communities, and ecosystems (Ratte, 1996). Although the ultimate level of concern may be populations, communities, or ecosystems, chemicals affect individual organisms, and the consequences of stress may be manifested at all levels of biological organization (Maltby, 1999). There is not a "right" level at which to study stress. Rather, different levels of organization provide information that, in combination, give insight into the effects of stress, their mechanistic bases, and their ecological and evolutionary consequences (Calow and Sibly, 1990).

Studies of populations and communities provide a description of the effects of stress, but they do not provide information on how effects are caused. Conversely, studies at the molecular and cellular level can provide detailed information on how chemicals interact with target sites but provide little or no information on the consequences of these effects for higher levels of organization. What is required is an integrated approach in which an understanding of the mechanistic bases of stress responses in individuals is used to predict or interpret their ecological consequences (Maltby, 1999).

Klok and de Roos (1996) used an individual-based model (Kooijman and Metz, 1984) to predict the effect of copper-induced changes in feeding and metabolism on the growth and reproduction of the worm *Lumbricus rubellus*. The model assumes that a fixed proportion of energy is spent on maintenance and growth, the remainder being allocated to reproduction. It also assumes that energy requirements for maintenance have priority over those for growth. Klok and de Roos (1996) investigated two different effects of toxic stress on energy budgets: (1) a decrease in energy assimilated, and (2) an increase in maintenance costs. The model predicted that, over the concentration range tested (13–362 mg Cu/kg), reproduction would be reduced by a toxicant-induced reduction in assimilation, but not by a toxicant-induced increase in maintenance costs. Both scenarios resulted in a decrease in individual growth. Predictions of individual performance were translated into population-level consequences using a size-structured matrix model. With both scenarios, populations exposed to the highest test concentration (i.e., 362 mg/kg) would become extinct, even though there was not effect of increased maintenance costs on reproduction. The reason for the population decline was the severe reduction in individual growth resulting from either reduced energy assimilation or increased maintenance costs. The

impairment of growth was such that animals would not attain reproductive size and therefore be incapable of reproducing. Using this approach, Klok and de Roos (1996) predicted copper concentrations at a critical level (i.e., population growth rate equals zero).

Understanding the energetic responses of individuals to stress is used to predict population-level effects. Knowledge of the mechanistic bases of toxicant stress is essential in order to understand why species differ in their susceptibility to stressors and how populations can persist in contaminated environments. Moreover, it is necessary to translate effects on individuals to effects on populations in order to evaluate the ecological consequences of stress. The effects of changes in feeding behavior are likely underestimated by ecologists when considering pesticide effects on aquatic organisms. Current toxicity tests focus on mortality (acute tests) and reproductive success (chronic studies) (Maltby, 1999). For many animals such as *Daphnia magna*, toxicant effects on feeding behavior may be associated with reproduction success and survival (Allen et al., 1999). The exposure to chemical stressors may increase energy expenditure due to the costs of defense and repair processes. However, it is clear from the results of a number of studies on a variety of species and stressors, that exposure to toxicants generally results in a decrease in feeding and hence in energy acquisition (Barata and Baird, 2000; William and Baird, 2002).

Stress-induced reductions in energy acquisition can predict the effects of toxicants on the growth, survival, and reproduction of individuals (Calow and Sibly, 1990; Barata and Baird, 2000). The toxic effect on *Daphnia* reproductive output has two components, which may be differently important for different chemicals. (1) A toxicant may slow down somatic growth and reproduction of the mother through “supply-side responses” or “demand-side responses” *sensu* Baird et al. (1990). Daphnids may need longer to produce their first clutch and remain smaller (i.e., have fewer eggs). This is the basis of Hanazato’s (1998) suggestion of using the linear growth of juvenile *Daphnia* as a surrogate for reproductive success. For example, Santojanni et al. (1998) discovered a strong and robust relationship between growth and fecundity of *D. magna* exposed to pyridine, cadmium, or chromium. They concluded that effects of toxicants on fecundity over 21 days can be predicted from linear growth during shorter exposure times.

1.3- Experimental system

The subjects introduced above were investigated testing the population-level responses of *Daphnia magna* Straus to a pulse and continuous contamination of the pyrethroid insecticide fenvalerate in two different food concentrations. In addition, the relationship between feeding and population-level responses of *D. magna* to a pulse contamination of fenvalerate was investigated.

1.3.1- Daphnia ssp.

Daphnia occupy a central role in pelagic food webs of temperate and arctic lakes and ponds (Fig. 6). Feeding on microalgae and bacteria, they can control phytoplankton biomass and species composition and influence seasonal succession of phytoplankton. When *Daphnia* are present, they often are the most important grazers (Gliwicz, 1990). They also affect the microbial food webs by feeding on flagellates and bacteria. At the same time they release nutrients and Dissolved organic matter (DOC), which enhances phytoplankton growth and bacterial production. On the other hand, *Daphnia* are a preferred food item for fish and invertebrate predators, i.e., daphniids are under strong top-down control (Rowe and Hebert, 1999).

Daphnia are cyclic or obligate parthenogens. Daphniids can be cultured as clonal lineages in the laboratory, providing genetically identical material for experimental work (Ebert et al, 1993). Daphniids are relatively easy to culture and handle. They are small enough to require minimal culture space, but large enough to be handled individually. Due to their parthenogenetic reproduction and the lack of larval stages, they have short generation times. Population growth rates can be as high as 0.3 to 0.4 d^{-1}, thus large numbers can be produced in a short period (Boersma, 1995).

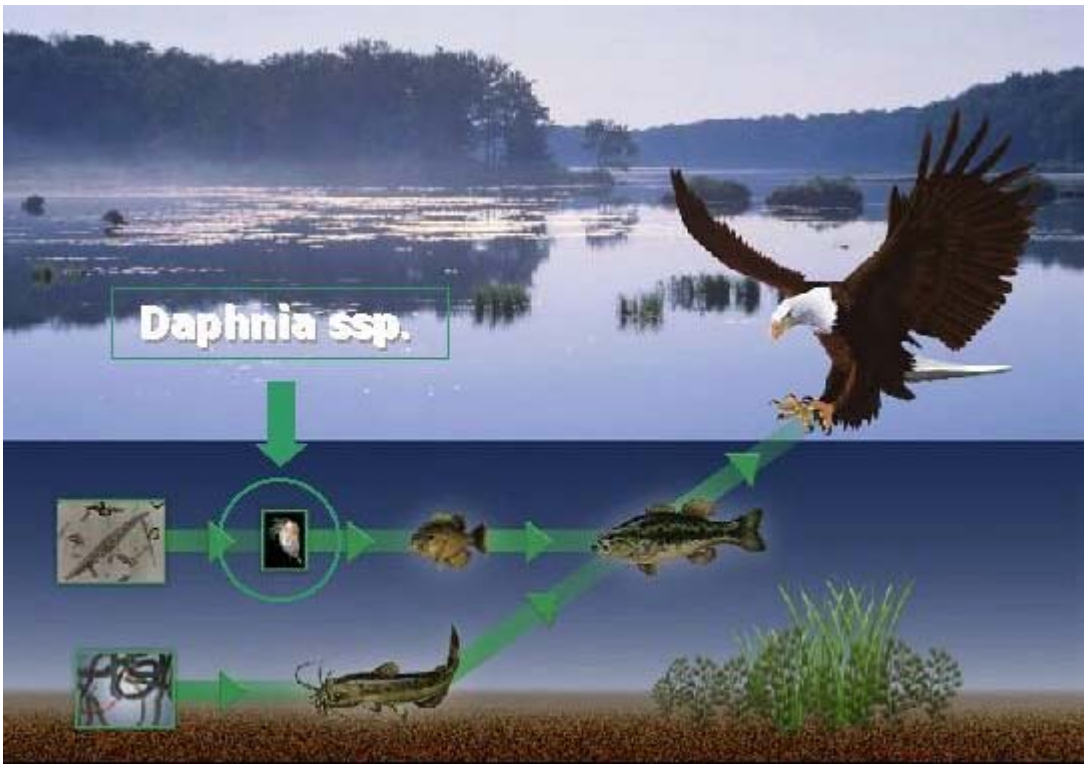


Fig. 6. Daphnia ssp. central role in pelagic food webs of temperate and arctic lakes. (Modified from U.S. Geological Survey, Ecology and Contaminants Project).

Daphnia carry their eggs and embryos in a transparent brood pouch on their backs until they hatch out. Egg numbers can be associated with individual mothers (Fig. 7). Therefore, *Daphnia* have been a favorite object for studies of population dynamics. Various types of population dynamics models have been developed for *Daphnia*, in particular new individual based population models (Gurney, et al., 1990).

*Daphnia´*s morphology and physiology have been studied extensively (Levy, 1959). There is much information on feeding, growth, respiration and excretion available to build general physiological models (McCauley et al 1990). In addition, the allocation of resources to growth and reproduction can be measured, which is the basis for life-history models (Kooijman et al.1995, Lampert and Trubetskova. 1996). The well-known physiology makes *Daphnia* species suitable object for ecotoxicology (Adema, 1978). *Daphnia* species show considerable phenotypic plasticity in morphology, life- history and behavior. They are usually used to measure reaction norms (OECD, 1998).

1.3.1.1- *Daphnia magna* Straus

The great waterflea lives in eutrophic fresh waters forming dense flocks. Oxygen stress increase the red color of these flocks because the increase of hemoglobin. Females usually reproduce parthenogenetically: diploid females produce diploid daughters without interference by the rare and much smaller males (Fig. 9) (Levi, 1959). Neonates have approximately a length of 0.8 mm, reproduction starts at 2.5 mm, and well-fed adult females can reach a length of 5 mm (Rowe, C.L. and Hebert, 1991). At 20°C, these crustaceans moult every 2-3 days, until death follows, usually after some months in the laboratory. In the field, *Daphnia magna* individuals typically live between 40 to 60 days (Rowe and Hebert, 1991).

Fig. 7. Rostrum and body of *Daphnia* female (Rowe and Hebert, 1991).

The first brood of neonates is released from adults' brood pouch typically at the age of 6-10 days, just before moulting. Shortly after moulting, new eggs are deposited in the brood pouch, so the incubation period almost equals the intermoult period (Ebert D., 1994). This means that females allocate most of their assimilates to offspring.

Daphnia magna individuals have a single large compound eye and a single small nauplius eye (Macagno et al 1973). The first pair of antennae is short and close to the rostrum ("nose"). The large second pair of antennae is used for swimming. The heart is rapidly beating in the "neck"-region. A paired caecum (blind ending digestive tubes) is visible just after the mouth. The maxillary gland (close to the mouth) is usually barely visible (Fig.7). The transition between the bright green algae in the foregut and the brown remains in the hindgut is sharp. A thick spine on the abdomen closes the brood pouch (Fig 7). The relative ease to culture these creatures on algae (Fig. 8), the ability to make clones, and their short generation time make “waterfleas” popular research subjects, especially in ecotoxicology (Adema, 1978).

Fig.8. *Daphnia magna* female with haploid eggs produced during the parthenogenic part of the its life cycle, *Daphnia magna* culture, and *Daphnia magna* neonates

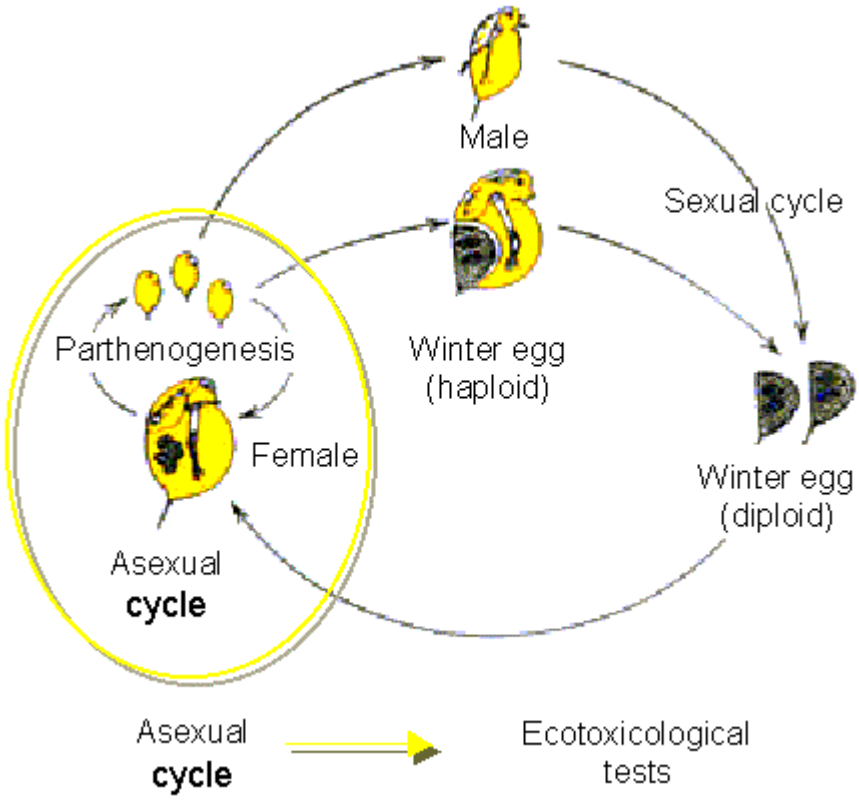


Fig. 9. *Daphnia* ssp. life-cycle in ecotoxicological tests.

1.3.1.2 Ecotoxicological tests with *Daphnia.*

Daphnia ssp. Acute Immobilization Test

The purpose of this acute immobilization test is the determination of the LC50, which is the estimated concentration to immobilize 50 % of individuals after 48 hours exposure. The 24-hour EC50 for immobilization can be also determined. The animals are considered immobilized when they are not able to swim for 15 seconds after gentle agitation of the test container. This standard test is described by the Guidelines for the Testing of Chemicals, Procedure Number 202 that was adopted by the OECD in April 1984 (Grandy, 1997).

Daphnia ssp. Reproduction Test

Since reproduction and development are two important criteria of ecosystem health, ideal life-history studies should last at least one life cycle. This means for *Daphnia*, to start when

neonates are 24h-old and to end when these neonates are in peak the reproduction. Therefore, the purpose of this reproduction test is to determine the effects of toxicants on survival and reproduction and other signs of intoxication (i.e. somatic growth and development). The exposure period lasts the same than the test resulting in a continuous exposure. This test is described by the Guidelines for the Testing of Chemicals, Procedure Number 211 that was adopted by OECD in September 1998 (OECD, 1998).

1.3.2- Synthetic Pyrethroids

The pyrethroid insecticides are typically esters of chrysanthemic acid having a high degree of lipophilicity (i.e. fat solubility). The original compounds in this series were the natural pyrethrins, which are isolated from the flowers of chrysanthemum. Pyrethroid chemistry and action are classified as Type 1 or Type 2, depending on the alcohol constituent. The Type 1 group is rather broadly defined. This group includes pyrethroids containing descyano-3-phenoxybenzyl or other alcohols. Many of the older nonphenoxybenzyl Type 1 compounds (e.g., pyrethrins, allethrin, tetramethrin) are unstable in the environment and this characteristic prevented their use in row crops. Introduction of the phenoxybenzyl (e.g., permethrin) or halogenated alcohols (e.g., tefluthrin) improved chemical stability and allowed the use of pyrethroids in the field. The Type 2 pyrethroids are more narrowly defined in terms of their chemical structure. They specifically contain an a-cyano-3-phenoxybenzyl alcohol, which increases insecticidal activity about 10-fold. Some commercially important Type 2 pyrethroids have altered the acid portion of the molecule to include a phenyl ring (e.g., fenvalerate) (Adelsbach and Tjeerdema, 2003).

Pyrethroids have been intensively studied by the wider scientific community. Therefore, an abundance of data exists with which to evaluate these compounds. As a result, registrants have been required to modify pyrethroid use labels to mitigate the perceived risk to aquatic ecosystems. Label restrictions include 25- and 150-ft no-spray distances for ground and aerial applications, respectively, when used directly adjacent to courses of water (Fig. 10). Other recommendations to reduce spray drift and runoff (thereby reducing exposure) also have been added to the use labels to promote safe uses (Solomon et al., 2001).

Fig.10. Pesticide application in order to mitigate the perceived risk to aquatic ecosystems, reducing spray drift and runoff towards adjacent courses of water (thereby reducing exposure to aquatic organisms). (60 Ways Farmers Can Protect Surface Water, This Land. On Line Publications Plus. College of Agricultural, University of Illinois).

The synthetic pyrethroid insecticides are widely used for pest management in both agriculture and public health. They have low toxicity to mammals and birds (LD50s[3] generally > 1,000 mg/kg) but are highly toxic to insects as well as some aquatic organisms, particularly aquatic insects (LC50s[4] generally < 10,000 ng/L) (Clark et al., 1989). The high lipid solubility results in a high octanol–water partition coefficient (>105) and a relatively great potential for bioconcentration into organisms from surrounding matrices such as water. Although pyrethroids may bioconcentrate in organisms, depuration is also rapid and bioaccumulation through the food chain is not a significant route for intoxication (Solomon et al., 2001).

Their low toxicity to mammals and birds also offers distinct advantages when they are used in agriculture but their toxicity to aquatic arthropods may present an environmental hazard if exposures are sufficiently great. Although the synthetic pyrethroids are generally considered to have low environmental persistence (water column $t_{1/2}$ < 4 d), they have longer environmental half-lives than the natural products from which they were developed ($t_{1/2}$ < 8 h in water, foliage, and so on), thus increasing their utility in agricultural use patterns (Adelsbach and Tjeerdema, 2003).

[3] LD50 Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
[4] LC 50 - median lethal concentration; the concentration of material that is estimated to be lethal to 50% of the test organisms

1.3.2.1- Mechanisms of action

The mechanism of action of the pyrethroids is through the nervous system (Fig 11). Their primary mode of action is through interference with ion channels in the nerve axon, resulting in hyperactivity of the nervous system with subsequent lack of control of normal function (Clark and Brooks, 1989). Two types of modes of action have been observed in mammals: 1- that associated with the type I pyrethroids (non–cyano-, non–halogen-substituted pyrethroids, e.g., pyrethrins, allethrin, tetramethrin) is characterized by tremors (T syndrome); 2- that associated with the type II pyrethroids (halogen-substituted acid moiety and cyano-substituted alcohol moiety, e.g., fenvalerate) is characterized by choreoathetotic writhing. The type II pyrethroids seem to have a primary mechanism of action at the presynaptic membrane that involves increased release of synaptic vesicles through an effect on voltage-dependent calcium channels. The symptomology of poisoning by type I and type II pyrethroids in invertebrates is not as distinct, similar depletion of presynaptic vesicles has been observed in insects (Clark and Brooks, 1989).

In addition to their action in the nervous system, pyrethroids have been reported to interfere with certain ATPase enzymes associated with maintaining ionic concentration gradients across membranes (Clark and Matsumura, 1982) (Fig. 11). This has been speculated to increase the sensitivity of freshwater aquatic organisms to these insecticides (Siegfried, 1993) through the addition of osmotic stress. This has also been suggested as the reason why fenvalerate is more toxic at median (isotonic) salinities in euryhaline species than at either high or low salinities (Hall and Anderson, 1995). In general, susceptibility to pyrethroids is dependent on sensitivity at the site of action and toxicokinetics. Included in the latter are bioavailability and rates of biological transformation. Haya (1989) suggested that biotransformation might play a role in differential toxicity of pyrethroids to fish. This same mechanism may explain the general lower sensitivity to pyrethroids of fish compared to arthropods.

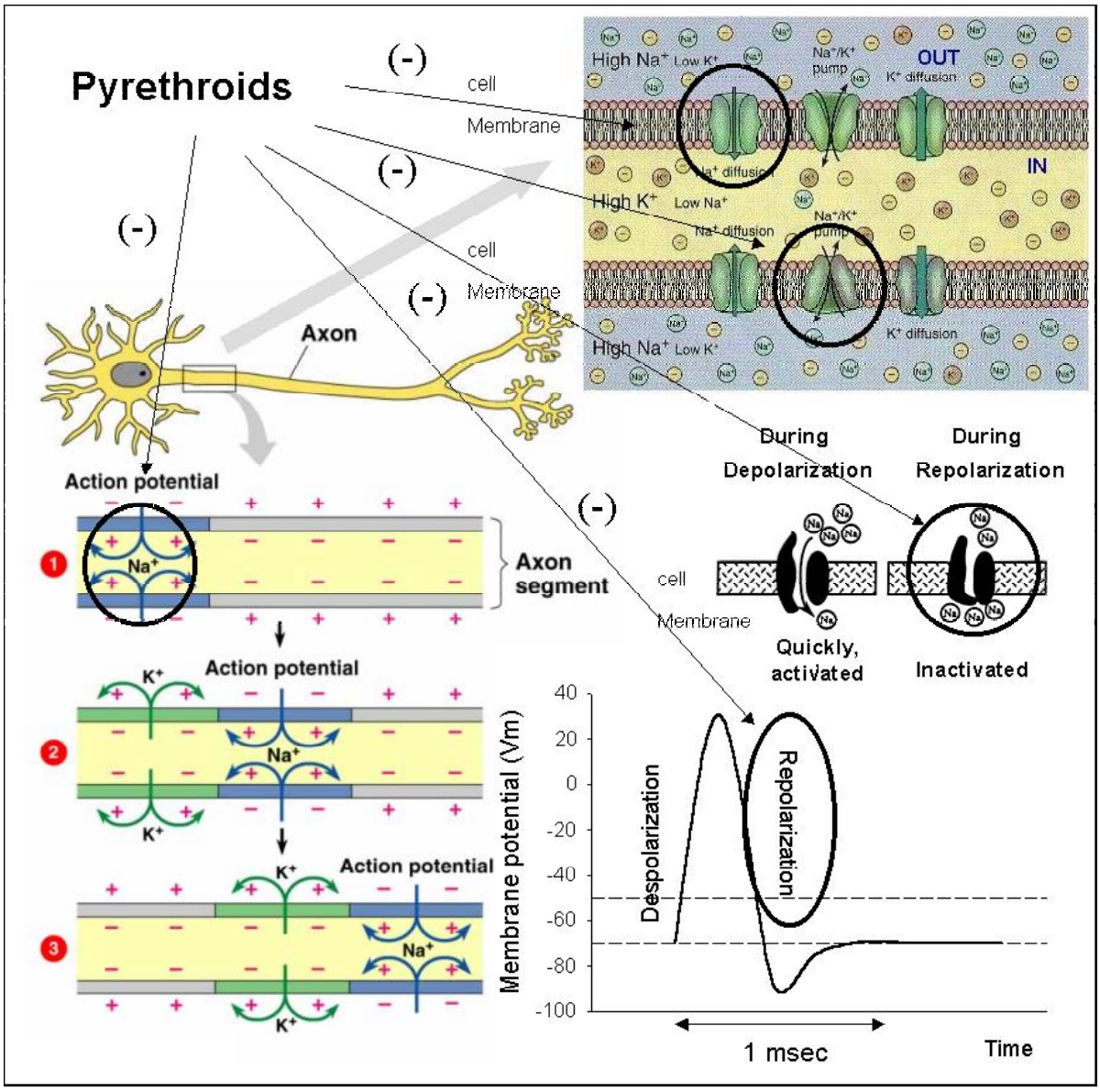


Fig. 11. The pyrethroids affected the sodium channels inhibiting the repolarization of the membrane (Clark and Brooks, 1989). Additional, pyrethroids seem to affected also the Na^+/K^+ ATPase affecting the ionic concentration gradients across membranes not only in the neurons (Clark and Matsumura, 1982).

1.3.2.2- Fenvalerate

Fenvalerate is a synthetic pyrethroid having no cyclo-propane ring in the molecule. It is prepared by the esterification of (2RS)-2-(4-chlorophenyl)-3-methylbutyric acid (also known as (2RS)-2-(4-chlorophenyl) isovaleric acid, CPIA, or Cl-Vacid) with (alphaRS)-alpha-cyano-3-phenoxybenzyl alcohol (Onho et al 1976). The molecular formula is $C_{25}H_{22}ClNO_3$. Fenvalerate has four stereoisomers because of the two chiral centres in the acid and alcohol moieties (Fig. 12). The compositions of the commercial products are racemic mixture of the four isomers in equal proportions (Table 1). In the present investigation, technical-grade fenvalerate containing 90-94% of fenvalerate was used.

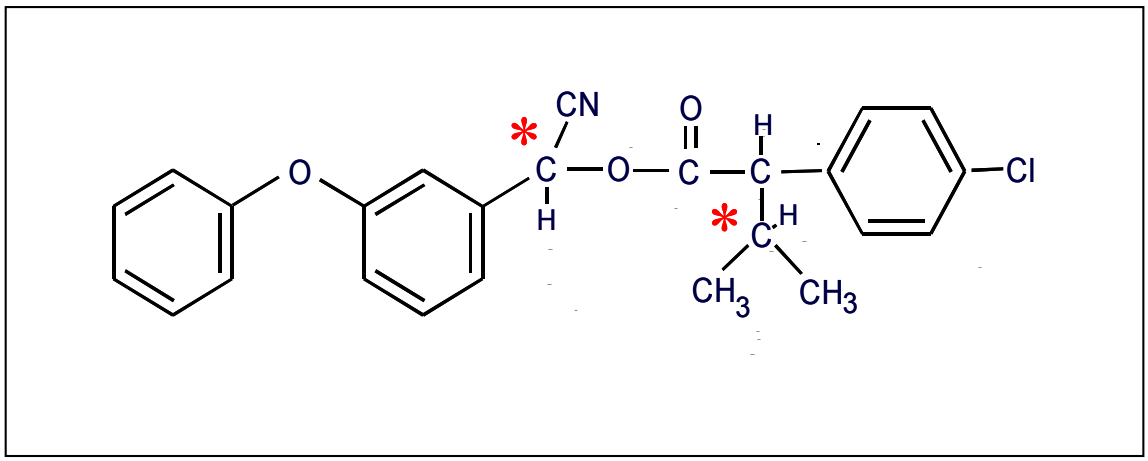


Fig. 12. Chemical structure of fenvalerate. The asterisks denote the chiral centers, which produce four stereoisomers.

Fenvalerate is stable to heat and moisture and is relatively stable (compared with natural pyrethrins) when exposed to light. It is more stable in acidic than in alkaline media, optimum stability being at pH 4 (Adelsbach and Tjeerdema, 2003). Fenvalerate is mostly employed in agriculture but also for insect control in homes and gardens and on cattle, alone or in combination with other insecticides. It is formulated as emulsifiable concentrate, ultra-low-volume concentrate, dust, and wettable powder.

Table 1. Some physical and chemical properties of fenvalerate

Physical state	Viscous liquid
Color	Yellow or brown
Relative molecular mass	419.9
Boiling point	300 °C at 4.93 kPa (37 mmHg)
Water solubility	< 2 µg/L
Solubility in organic solvents	Soluble
Relative density (25 °C)	1.175
Vapor pressure (25 °C)	0.037 mPa
Log octanol-water partition coefficient (log P_{ow})	6.2

1.3.2.2.1-Toxicity to aquatic invertebrates

Non-target invertebrates, except molluscs, are more susceptible to fenvalerate than fish, the LC50 for this group ranging from 0.08 to 2 µg/L (Solomon et al, 2001). Fenvalerate is relatively non-toxic to oysters and algae (LC50 >1000 µg/L) over short exposure periods. Snails (*Heliosoma trivolvis*) exposed for 28 days to 0.79 µg/L, the highest concentration tested, showed no change in behavior or survival (Anderson, 1982).

In contrast, fenvalerate affected survival and feeding rate of cladoceran and one species of calanoid (Day and Kaushik, 1987a). The 48-h LC50 values for cladoceran were: 2.52 µg/L for adult *Daphnia magna*, 0.83 µg/L for *D. magna* aged 48 h (or less); 0.29 µg/L for adult *Daphnia galeata mendotae*; 0.21 µg/L for adult *Ceriodaphnia lacustris*; 0.16 µg/L for *D. galeata mendotae* aged 48 h. *Diaptomus oregonensis* was the most sensitive species with a 48-h LC50 of 0.12 µg/L. Rates of filtration of algae were reduced at sub-lethal concentrations of fenvalerate. *Ceriodaphnia lacustris* was the most sensitive species; with rates of filtration significantly decreased at fenvalerate concentrations of 0.01 µg/L (Day and Kaushik, 1987a).

In addition, Day & Kaushik (1987b) conducted life-cycle studies on the toxicity of fenvalerate to *Daphnia galeata mendotae.* Life-table response experiments (LTRE) were applied in order to investigate the effects of fenvalerate on population dynamic of *Daphnia galeata mendotae*. At a concentration of 0.05 µg/L, fenvalerate increased significantly longevity from 37.6 to 51.6 days. However, at the same concentration, the offspring production was decreased.

Higher concentrations of fenvalerate resulted in a survival reduction. The population growth rate (r) was reduced at a concentration of 0.5 µg/L (Day & Kaushik, 1987b)

In outdoor microcosms, fenvalerate dissipated rapidly during the first 24 h. However, the density of crustaceans decreased and this was followed by an increase of small zooplankton and phytoplankton density, likely because the reduction of competition and grazing (Day et al., 1987 and Woin et al., 1998). In addition, 1-h pulse of fenvalerate resulted in a decrease of survival and a delay in emergence in the caddisfly, *Limnephilus lunatus*, several weeks following exposure when animals were placed in outdoor microcosms (Liess and Schulz, 1996). In field studies, fenvalerate exposure associated to agriculture runoff decreased the densities of the grass shrimp *Palaemonetes pugio* (Baughman et al., 1989), of the caddisfly *Limnephilus lunatus* and of *Gammarus pulex* (Schulz and Liess, 1999).

Table 2. Acute toxicity of fenvalerate to non-target freshwater organisms

Species	Size[a]	Parameter	Toxicity (µg/L)	Formulation[b]	System[c]	T (° C)	Reference
Gammarus pseudolimnaeus	Adult-juv.	96-h LC_{50}	0.03	T	F	15	1
Gammarus pseudolimnaeus	1-3mm-	96-h LC_{50}	0.05	T	R	17	1
Daphnia magna	1st instar	96-h LC_{50}	0.032	T	S	17	2
Midge *Chironomus pulmosus*	3rd instar	48-h LC_{50}	0.43	T	S	22	2
Mayfly *Ephemerella sp.*	Larva	9-day LC_{50}	0.08	T	F	15	1
Rhagionid fly *Atherix*	Larva	28-day LC_{50}	0.03	T	F	15	1
Stonefly *Pteronarcys dorsata*	Naiad	72-h EC_{50}	0.13	T	F	15	1
Stonefly *Nitocra spinipes*	3-6 weeks old	96-h LC_{50}	1.9	EC	S	17	3

References: 1-Anderson, 1982; 2-Mayer and Ellersieck, 1986; 3-Linden et al., 1979
[a] juv = juvenile.
[b] T = Technical, EC = Emulsifiable concentrate.
[c] R = Renewal, S = Static, F = Flow-through.
[d] expressed as mg CaCO3 per liter.

1.3.2.2.1-Persistence in Water

Fenvalerate in buffered aqueous solution was stable at pH 5.0 and 7.0 (half-lives of 130-220 days), while at pH 9.0 it underwent hydrolysis (half-lives of 64.6-67.2 days) mainly via ester bond cleavage (Agnihotri et al., 1986). The main product was 2-(4-chlorophenyl)-3-methylbutyric acid, which amounted to 14.9% of the applied ^{14}C after 28 days. The persistence of fenvalerate has been evaluated in water and sediment contained in open trenches (3 m x 1 m x 30 cm) lined with alkathene sheet. The insecticide emulsion was sprayed on the surface of the water at the normal rate and at twice the recommended dosage, and the dissipation from water was rapid about 74-80% of the pesticide was lost within 24 h at both application rates (Agnihotri et al., 1986).

CHAPTER II

Response and recovery of *Daphnia magna* Straus to the insecticide fenvalerate: Relevance of exposure duration.

Abstract- This study compares the demographic responses of *Daphnia magna* Straus exposed to fenvalerate continuously (21d) and as a pulse (24h). Survival was more severely reduced in the continuous than in the pulse exposure regime, as expected from the outcomes of previous investigations: Complete mortality was elicited by 1 µg/L when present continuously and 3.2 µg/L in the pulse. For reproductive endpoints the results indicate that pulse exposure to fenvalerate exerts transient effects on reproduction at concentrations similar to those for continuous exposure: At the beginning of the reproductive phase (day 10), a significant reduction in number of offspring was observed at 0.3 and 0.1 µg/L for continuous and pulse exposure, respectively. After 21 days, however, effects of continuous and pulse exposures differed markedly. Following pulse exposure, effects on reproduction recovered (close to control values), over a broad range of concentrations from 0.1 up to 1 µg/L. In the continuous exposure regime, no substantial recovery was observed.

Key words – Pulse exposure, Toxicant, Population growth rate, Recovery, *Daphnia magna*.

2.1- INTRODUCTION

Fenvalerate is characterised by a low water solubility, log k_{ow} = 6.2, and strong sorptive properties, both of which are expected to reduce its bioavailability in natural environments (Adelsbach and Tjeerdema, 2003). The high lipid solubility results in a great potential for bioconcentration into organisms from surrounding matrices such as water. Although pyrethroids may bioconcentrate in organisms, depuration is also rapid Consequently, studies with continuous exposures may overestimate the effects of pyrethroids in the field (Maund et al., 2001).

The comparison between effects of continuous and pulse exposure to pyrethroids on crustacean have not been well characterized with respect to chronic responses and recovery.

Therefore, the main objective of this study was to evaluate these effects by exposing *Daphnia magna* Straus to fenvalerate, either continuously (21d) or as a pulse (24h).

2.2- MATERIALS AND METHODS

2.2.1- *Daphnia magna* culture

Continuous cultures of *Daphnia magna* Straus (clone B, Bayer, Germany) were maintained at a density of 10 adults/L in M7-Elendt medium at 20°C ± 1°C in a light-dark regime of 16:8h, with light intensity of ~15 µmol/m^2/s (OECD, 1998). The conductivity, pH and oxygen concentration were 630 µS/cm, 7.4 and 7.15 mg/L, respectively. Once every week a new culture was initiated using <24-h-old neonates from a three-week-old culture. The newly released neonates were discarded twice a week, and the medium was renewed three times a week at regular intervals. The animals were fed with a suspension of batch-cultured green microalgae (*Desmodesmus subspicatus*), which were cultured in algae medium according to Grimme and Boardman (1972). The algae were harvested in exponential growth phase, centrifuged, and the pellet resuspended in Elendt-M7 medium in desired dilutions. The animals were fed three times a week with equivalent daily rations of ~0.045 mgC/*Daphnia*/day for the one-week-old animals and ~0.07 mgC/*Daphnia*/day for the older animals.

2.2.2- Fenvalerate exposure and measurement

Fenvalerate, (RS)-α-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate (CAS: 51630-58-1), was obtained from Riedel-de Haën®, Seelze, Germany (99.9%). The carrier solvent DiMethylSulfOxide (DMSO, Merck®, Darmstadt, Germany, 99.8%) was used to add fenvalerate to the test medium. A stock solution of 0.1 mg/L was prepared using Elendt-M7 medium before the start of each experiment. The maximum amount of DMSO was 0.00003% (v/v). Six different nominal concentrations were used for both exposure regimes (control, 0.03, 0.1, 0.3, 0.6 and 1 µg/L). For pulse exposure a higher concentration, 3.2 µg/L, was also tested. The animals were fed during exposures in both regimes, the alga suspension being added to fenvalerate solutions before the *Daphnia* individuals were introduced.

Actual exposure concentrations were only determined for 0.6, 1 and 3.2 µg/L test solutions due to detection limits. Samples were measured in the Institute of Ecological Chemistry

and Waste Analysis, Technical University of Braunschweig, Germany. Solid-phase extraction of 1-L volumes was carried out using C18-columns (Baker, Phillipsburg, NJ, USA). The analyses were performed by means of gas chromatography/mass spectormetry applying electron impact ionisation and selected ion monitoring mode (Agilent 6890 Series GC System with Agilent 7683 Series Injector and Agilent Network Mass Selective Detector; all Agilent, Waldbronn, Germany). Analytical measurements of the 0.6, 1 and 3.2 µg/L test solutions at t = 1h showed a reduction in the nominal concentrations of 50-60% (0.2 ±0, 0.43 ±0.12, and 1.47 ± 0.28 µg/, respectively). The actual concentration of the 1.0 µg/L test solution decreased to 0.37±0.06 µg/L after 24h. Nominal concentrations are given in the following sections.

2.2.3- Life table response experiments

Standard *Daphnia* reproduction tests (OECD, 1998) (Fig. 1) were performed with two exposure regimes: pulse and continuous exposures. In the pulse exposure regime, the animals were only exposed during the first 24h (Fig. 2). In the continuous exposure, regime fenvalerate was renewed three times a week together with the medium (Fig. 2). In both regimes, 15 neonates (<24h) per concentration were individually exposed in presence of food (0.07 mgC/*Daphnia*/day). This food level was chosen in order to meet standard requirements (>60 neonates in the control treatments) (Grimme, and Boardman, 1972). Other experimental conditions were the same as those described for the *Daphnia* culture. Every day, dead and newborn animals were counted and removed. The mean number of neonates per female was calculated by the multiplication of the number of brood times the mean brood size. The life-history traits were integrated in the population growth rate (i.e. intrinsic rate of natural increase) on days 10 and 21. Values were calculated according to the Euler equation (1)

$$\sum_{x=0}^{x} l_x \cdot m_x \cdot e^{-r \cdot x} = 1 \qquad (1)$$

Where r = per capita rate of increase for the population (number per day), x = age of class (days; 1, 2, 3…a), a= oldest age class in the population (10 and 21 days in the present study), l_x = probability of surviving at age x, and m_x = fecundity at age x.

Because this calculation involves a summation over several age classes, r cannot be isolated on one side of the equation to provide a closed-form, algebraic solution. Instead, interactive calculations must be performed in order to determine an r value that satisfies Eq. 1. Variance associated with r values for statistical comparison was estimated using the Jackknife method as described by Meyer et al. (1986).

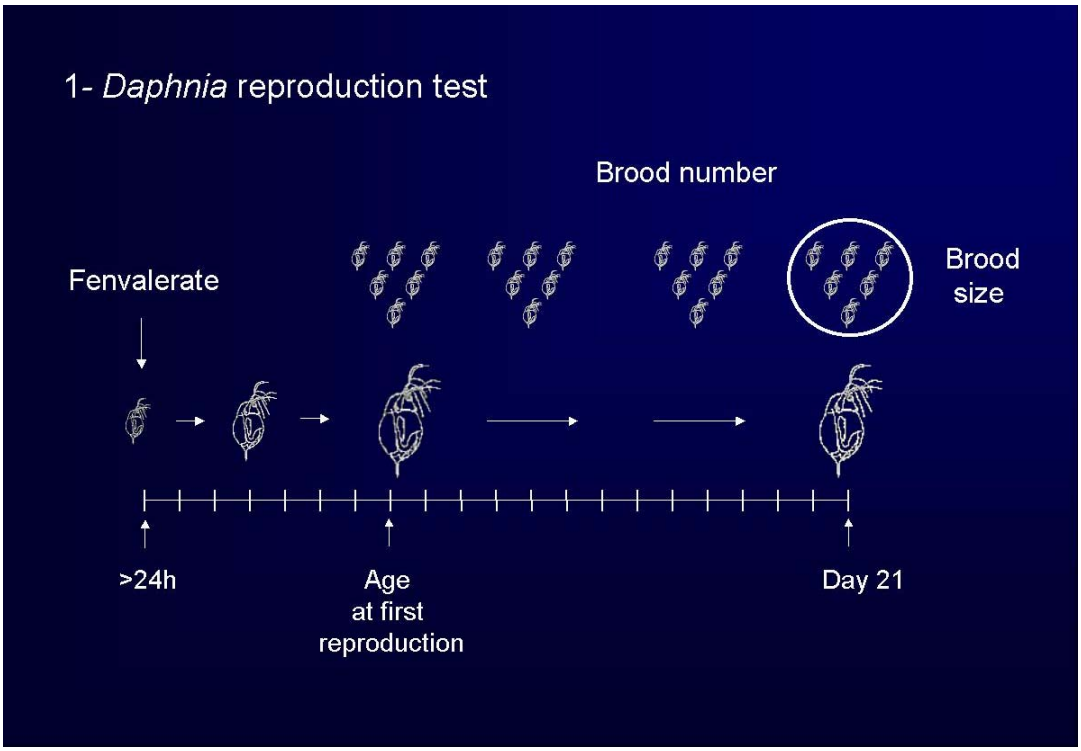


Fig. 1. *Daphnia magna* reproduction test

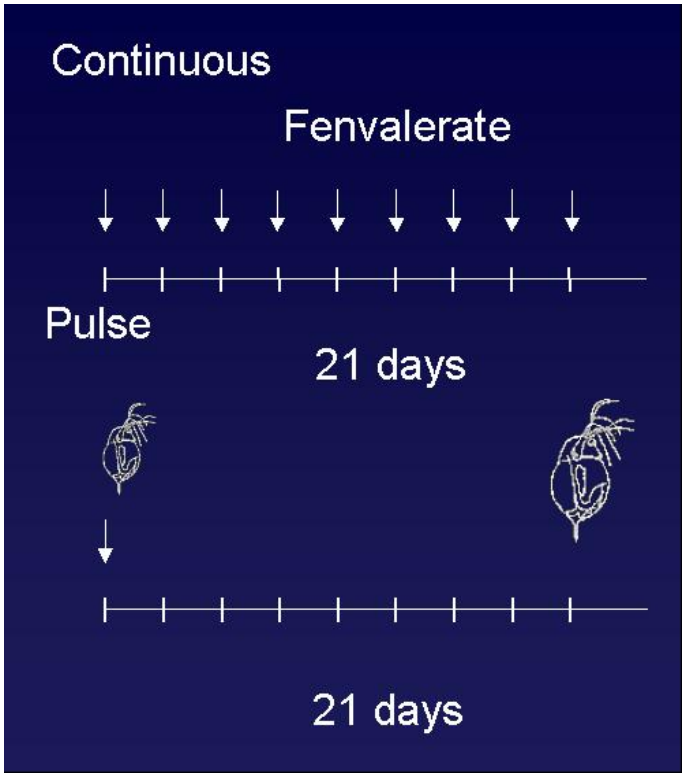


Fig. 2. Pulse and continuous exposure

2.2.4- Statistical analysis

The data were tested for normality (Kolmogorov-Smirnov test, $p < 0.05$) and homogeneity of variances (Levene's test, $p < 0.05$). When these requirements were not met, rank transformation was chosen (Potvin and Roff, 1993). The influence of the exposure duration on fenvalerate toxicity to *D. magna* was evaluated by applying two-ways analysis of variance (ANOVA) for the mean age at first reproduction, brood number, brood size, and population growth rate. Due to the complete mortality in the continuous level, the concentration level of 1 µg/L was excluded for the two-way analysis of variance. Repeated measures analysis of variance ($p < 0.05$) was applied in order to evaluate the progression of the concentration-response over time. Empty cells due to mortality were filled with the mean value of the level in order to avoid unbalance design. The fenvalerate treatments were compared with controls by the Dunnett test ($p < 0.05$).

2.3- RESULTS

2.3.1- Survival

After 2 days, all individuals survived in the control treatments, as well as with 0.1 and 0.3 µg/L of fenvalerate in continuous and pulse exposure, respectively (Fig. 3). Significant effects were observed at 3.2 µg/L in the pulse exposure treatment. After 10 days, significant mortality had occurred at 0.6 and 1 µg/L in the pulse and continuous regimes, respectively, and mortality was complete at 1 µg/L with continuous exposure, and 3.2 µg/L in the pulse regime. Repeated measures analysis of variance showed that the concentration-response relationship changed significantly between days 2 and 10, but not between days 10 and 21 in both exposure regimes. This indicated that changes in mortality were present only in the first half of the experiment for both exposure regimes (Two-way ANOVA, p<0.05). Although more individuals survived at concentrations higher than 0.3 µg/L with the pulse exposure, the slope of response to fenvalerate was similar for both regimes (Fig. 3).

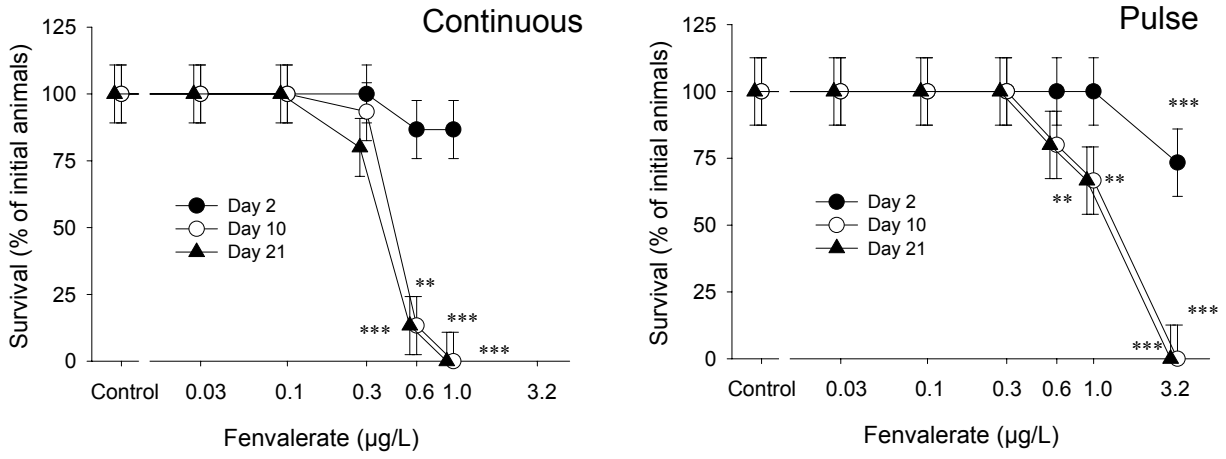


Fig. 3. Effects of continuous and pulse exposure to fenvalerate on the survival at day 2, day 10 and day 21, as % of initial number of individuals. For clarity, mean values at day 21 are slightly shifted to the left side for all concentration levels in both exposure regimes. Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

2.3.2- Reproduction

Fenvalerate increased the age to first reproduction (Fig. 4), but did not reduce the brood size (Table 1). After 10 days, fenvalerate significantly inhibited reproduction at concentrations as low as 0.3 and 0.1 µg/L with continuous and pulse exposure, respectively (Fig. 5). However, after 21 days, the absence of significant effects indicated a recovery of reproduction in the pulse exposure regime over a broad range of concentrations below 3.2 µg/L. In contrast, with continuous exposure recovery was only observed at 0.3 µg/L. Repeated measures analysis of variance (p<0.05) showed that the concentration-response relationship changed significantly between days 10 and 21, only for the pulse exposure regime. This indicated that the recovery altered the concentration-response relationship (Fig. 5).

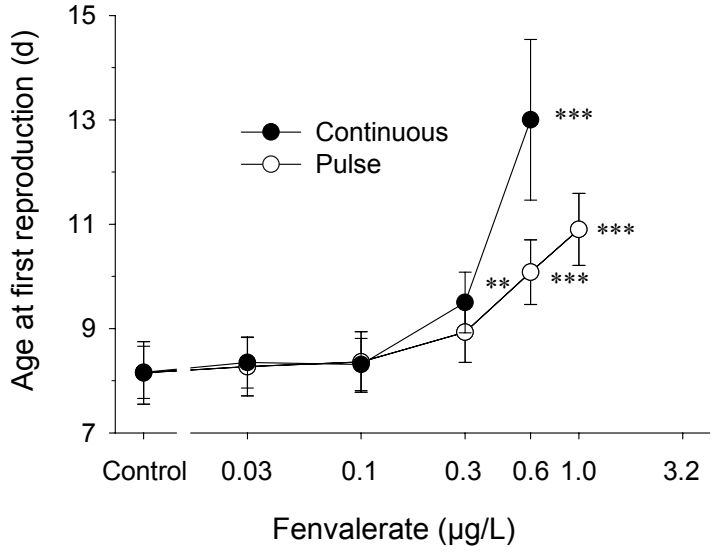


Fig. 4. Effects of continuous and pulse exposure to fenvalerate on the age at first reproduction. Error bars indicate 95% confidence limit. Asterisks indicate significant (Two-way ANOVA, Dunnett test: $^{**}p < 0.01$; $^{***}p < 0.001$) differences from control treatments.

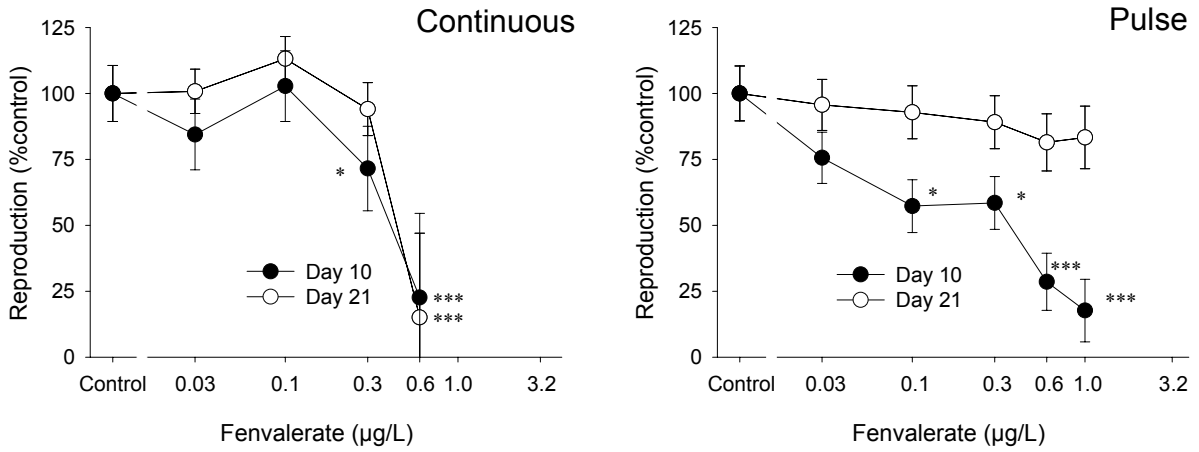


Fig. 5. Effects of continuous and pulse exposure to fenvalerate on reproduction as % of the mean of neonates per living female in control treatments at day 10 and 21. Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

Table 1. Effects of continuous and pulse exposure to fenvalerate (FV) exposures on number of broods per living female and brood size (i.e. number of offspring per brood) at day 21. Mean ± 95% confidence limit.

Exposure	FV (µg/L)	Number of broods	Brood size
Continuous	Control	4.84±0.25	12.68±0.88
	0.03	4.85±0.25	12.76±0.86
	0.1	4.95±0.25	13.96±0.88
	0.3	4.26±0.21 **	13.37±0.74
	0.6	2.00±0.79 ***	04.75±1.917 *
	1.0	–[a]	–[a]
Pulse	Control	4.86±0.30	12.37±1.03
	0.03	4.70±0.29	13.25±0.99
	0.1	4.79±0.31	12.50±1.03
	0.3	4.57±0.31	12.60±1.03
	0.6	4.33 ±0.32 *	12.15±1.11
	1	4.30 ±0.35 *	12.62±1.21
	3.2	–[a]	–[a]

[a] No values were calculated due to complete mortality. Asterisks indicate significant (Two-way ANOVA, Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

2.3.3- Population growth rate

After 10 days, significant effects were observed at 0.3µg/L for both exposure regimes (Table 2). In the continuous regime, no values were calculated at 0.6 µg/L due to an apparent delay in the age at first reproduction. The complete mortality did not allow values to be calculated for 1 and 3.2 µg/L with continuous and pulse exposure, respectively. After 21 days, in the continuous regime a negative value of population growth rate was calculated at 0.6 µg/L. In the pulse regime, the differences from control values remained significant at 0.6 and 1 µg/L; nevertheless, the recovery of reproduction diminished the magnitude of the inhibition (Table 2). Accordingly, repeated measures analysis of variance ($p < 0.05$) showed that the concentration-response relationship changed significantly between days 10 and 21 only for the pulse exposure regime in the similar ways that observed for the reproduction (Table 2).

Table 2. Effects of continuous and pulse exposure to fenvalerate on the combination of reproduction and survival in the intrinsic rate of natural increase or population growth rate (d^{-1}). Mean ± 95% confidence limit.

Fenvalerate	Continuous exposure		Pulse exposure	
(µg/L)	Day 10	Day 21	Day 10	Day 21
Control	0.287±0.035	0.288±0.01	0.324±0.042	0.293±0.012
0.03	0.341±0.034	0.296± 0.01	0.302±0.039	0.285±0.016
0.1	0.305±0.034	0.294± 0.01	0.266±0.041	0.278±0.012
0.3	0.214±0.041**	0.268±0.012*	0.251±0.041*	0.272±0.012
0.6	-[b]	-0.004±0.026***	0.157±0.044***	0.244±0.013***
1	–[a]	–[a]	0.108±0.048***	0.235±0.014***
3.2	–[a]	–[a]	–[a]	–[a]

No values were calculated due to [a] complete mortality or [b] marked delay of first reproduction. Asterisks indicate significant differences from control treatments. (Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

2.4- DISCUSSION

The responses of survival, reproduction and population growth rate were compared at low (sublethal) (0.03-0.3µg/L) and high (lethal) (0.6-3.2µg/L) concentrations of fenvalerate. Such concentrations have been reported during the peak growing season for vegetable crops in surface waters of agricultural areas (Baughman et al. 1989), whereas short-term peak concentrations of 0.8 - 6 µg/L were measured in agricultural streams during runoff events (Liess et al, 1999; Schultz and Liess, 1999; Scott et al., 1999). Therefore, the concentration tested in the present work can be considered an environmentally realistic concentration of fenvalerate.

Survival was reduced to a greater extent in the continuous than in the pulse exposure regime, as was to be expected from the outcomes of previous investigations (Reinsert et al., 2002). Mortality occurred mainly until day 10 in both regimes (Fig. 3), due to a higher sensitivity of the early life-stages to fenvalerate (Day and Kaushik 1987a). Age to first reproduction was delayed for both exposure regimes (Fig. 4) and hence reproduction and population growth rate were decreased following exposure to fenvalerate (Fig. 5; table 2). A similar observation was reported for the caddisfly *Limnephilus lunatus* when exposed to a 1-h pulse of fenvalerate (Liess and Schulz, 1996).

When comparing pulse exposure and continuous exposure at day 10, the inhibition in reproduction and decrease in population growth rate were found at a similar or even lower concentration in the pulse exposure. These results suggest that at low concentrations the fenvalerate toxicity is more related to the concentration peak, rather than to the exposure duration. Similar results were found for the fenvalerate toxicity to caddisfly larvae, since exposure to 1µg/L for 1 h caused more pronounced effects (i.e. emergence delay) than did 10-h exposure to 0.1 µg/L (Schulz and Liess, 2000). Correspondingly, Naddy et al (2000) concluded that the magnitude of the contamination is a greater determining factor of chlorpyrifos toxicity to daphnids than exposure duration.

Nevertheless, recovery from sublethal effects could be observed in the pulse exposure regime but only to a minor extent in the continuous exposure regime: on day 21, reproduction was severely reduced and the population growth rate was negative in the continuous regime (0.6 µg/L, Table 2). According to Stark and Walthall (2003) a negative population growth rate may predict the extinction of the population. In contrast, following pulse exposure both reproduction and population growth rate recovered for a range of concentrations from 0.1 to 1 µg/L. In view of

the lack of recovery in the continuous exposure regime, it follows that a concentration of fenvalerate sufficient to cause the extinction of a population exposed in the long term may be more than 5 times lower than that found for the pulse exposure regime.

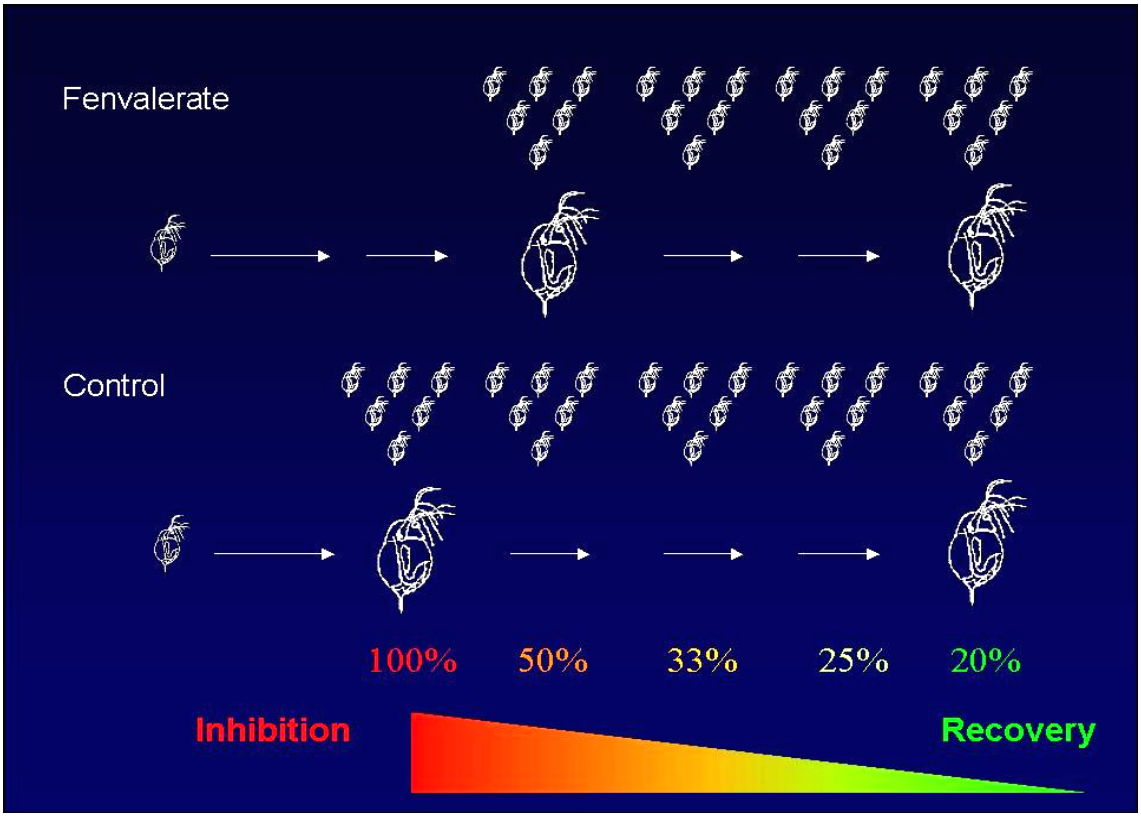


Fig. 4. Recovery of the inhibition produced by the delay in the first reproduction. The inhibition was buffered by the increase of released brood, since fenvalerate did not affect the brood size.

2.5- CONCLUSION

The results of this study showed clearly that exposure duration influences fenvalerate toxicity to *D. magna.* In addition, recovery from sublethal effects should be taken into account when predicting responses on the population level (r) short-term exposure. When predicting responses on the community level, however, a transient reduction in fitness might be of relevance when competitive and predatory pressure from other species is present.

CHAPTER III

Exposure duration under low food: its influence on responses and recovery of *Daphnia magna* Straus to fenvalerate

Abstract: This study compares the demographic responses of *D. magna* following continuous (21d) and pulse (24h) exposure to the pyrethroid fenvalerate under low food concentration. The great differences between exposure regimes were observed in survival that was only affected before maturity (i.e. juvenile survival). Significant mortality occurred at 0.1 with the continuous exposure and 0.3 μg/L with continuous and pulse exposure; and 100% mortality was reached at 0.6 and 3.2 μ/L with continuous and pulse exposure, respectively. In both regimes, fenvalerate exposure retarded the somatic growth delaying the first reproduction. This reduced the number of broods per female, which produced a severe inhibition of the number of neonates per female and the population growth at beginning of the reproduction. However, initial inhibition was partially compensated after day 21 by the progress of the reproduction. Nevertheless, the population growth rate on day 21 was highly correlated with the mortality. In addition, the decomposition analysis confirmed that mortality was the main contribution to the inhibition of the population growth rate on day 21 in both exposure regimes. It is worth to notice that these sublethal effects (i.e. developmental delay) would be more relevant in case of semelparous species, because the buffering mechanism cannot take place in these species. The elasticity analysis showed that the population growth rate in the continuous exposure was more sensitive to changes the survival juvenile than in the pulse exposure. Fenvalerate effects on survival in the pulse exposure were lessened at population-level.

Key words: Exposure duration, conditions, iteroparous species, Elasticity, *Daphnia magna*.

3.1- INTRODUCTION

Food concentration is of major importance for the physiological state of the organism; therefore, low food concentration may reduce resources available for defense against environmental stress and hence increase susceptibility to toxic stress (Sibly, 1999). On the other hand, the alga concentration (i.e. food) can modify the bioavailability of organic pollutant depending their water solubility (Barry et al., 1995; Rose et al., 2002).. The pyrethroid fenvalerate used in this study is a hydrophobic insecticide (log k_{ow}= 6.4) (Adelsbach and

Tjeerdema, 2003). Day and Kaushik (1987c) observed that the amount of fenvalerate adsorbed to the algae increases with food (i.e. alga) concentration. However, this did not result in an increased of fenvalerate bioconcentration.

Recent studies compare the chronic responses of *Daphnia magna* to continuous and pulse exposure to insecticides (Hosmer et al., 1998; Naddy, et al., 2001). In these studies, however, comparisons are carried out considering only effects on individual-level traits (i.e. survival, age at first reproduction, reproductive output) and low-food concentration conditions were not considered. A more relevant measure of ecological impact can be provided by the population growth rate ($r = \log_e \lambda$), which integrates individual-level traits describing changes in population density through time (Ratte, 1996; Calow et al., 1997).

The extent to which population growth rate responds to toxicant exposure depends on the severity of the toxicant effects on the individual life-history traits as well as on the sensitivity of population growth rate to changes in each of the individual-level traits contributing to it (i.e. survival, reproductive output, age to first reproduction) (Caswell, 1996). Simulation and analytical studies suggest that effects of toxicant at the population-level (i.e. population growth rate) may be less than or equal sensitive to changes in individual-level traits when populations are close to their current capacity. Therefore, risk assessments based on individual-level traits would be overprotective in predicting the impact of toxicants at population-level under low-food concentrations (i.e. current capacity), in particular for iteroparous species such as *D. magna* (Calow et al., 1997; Forbes et al., 2001).

In order to verify the above mentioned suggestions and the influence of the exposure duration on them, the main objective of this experimental study is to compare responses of individual-level traits and their extrapolation to population-level following continuous (21d) and pulse (24h) exposure to fenvalerate under food restriction.

3.2- MATERIALS AND METHODS

Daphnia magna culture

Continuous cultures of *Daphnia magna* Straus (clone B, Bayer, Germany) were maintained at a density of 10 adults/L in M7-Elendt medium at 20°C ± 1°C in a light-dark regime of 16:8h, with light intensity of ~15 µmol/m^2/s (OECD, 1998). Oxygen concentration, pH, conductivity were 7.15 mg/L, 7.4 and 630 µS/cm, respectively. Once every week a new culture was initiated using <24-h-old neonates from a three-week-old culture. The newly released neonates were discarded twice a week, and the medium was renewed three times a week at regular intervals. The animals were fed with a suspension of batch-cultured green microalgae (*Desmodesmus subspicatus*), which were cultured in algae medium according to Grimme and Boardman (1972). The algae were harvested in exponential growth phase, centrifuged, and the pellet resuspended in Elendt-M7 medium in desired dilutions. The animals were fed three times a week with equivalent daily rations of ~0.045 mg carbon/*Daphnia*/day for the one-week-old animals and ~0.07 mg carbon/*Daphnia*/day for the older animals.

3.2.1- Fenvalerate exposure and measurement

Fenvalerate, (RS)-α-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate (CAS: 51630-58-1), was obtained from Riedel-de Haën®, Seelze, Germany (99.9%). The carrier solvent DiMethylSulfOxide (DMSO, Merck®, Darmstadt, Germany, 99.8%) was used to add fenvalerate to the test medium. The maximum amount of DMSO was 0.00003% (v/v). Seven different nominal concentrations were used for both exposure regimes (0, 0.03, 0.1, 0.3, 0.6, 1 and 3.2 µg/L). The animals were fed during exposures in both regimes, the algae suspension being added to fenvalerate solutions before the *Daphnia* individuals were introduced. Actual exposure concentrations could only be determined for 0.6, 1 and 3.2 µg/L test solutions (n = 3) at t = 1h due to the detection limits. Additionally, a 1 µg/L test solution (n = 3) was measured at t = 24h. Samples were measured in the Institute for Ecological Chemistry and Waste Analysis, Technical University of Braunschweig, Germany. Solid-phase extraction of 1-L volumes was carried out using C18-columns (Baker, Phillipsburg, NJ, USA). The analyses were performed by means of gas chromatography/mass spectormetry applying electron impact ionisation and selected ion monitoring mode (Agilent 6890 Series GC System with Agilent 7683 Series Injector and Agilent Network Mass Selective Detector; all Agilent, Waldbronn, Germany). Analytical measurements

of the 0.6, 1 and 3.2 µg/L test solutions at t = 1h showed a reduction in the nominal concentrations of 50-60% (0.2 ±0, 0.43 ±0.12, and 1.47 ± 0.28 µg/, respectively). The actual concentration of the 1.0 µg/L test solution decreased to 0.37±0.06 µg/L after 24h. Nominal concentrations are given in the following sections.

3.2.2- Life table response experiments

Standard *Daphnia* reproduction tests (OECD, 1998) were performed with two exposure regimes: pulse (24h) and continuous exposures (21 d). In the pulse exposure regime, animals were only exposed during the first 24h. In the continuous exposure regime, fenvalerate was renewed three times a week together with the medium. In both exposure regimes, 15 neonates (<24h) per concentration were individually exposed in presence of food (~ 0.02 mg C/*Daphnia*/day). Low-food conditions in this study are defined as 1/3 of the necessary food to obtain at least 60 neonates per female in control treatments (OCDE, 1997). Other experimental conditions were the same as those described for the *Daphnia* culture. Every day, dead and newborn animals were counted and removed and. Age at first reproduction, brood number, and brood size were determined from the number of living neonates recorded daily. The size was measured from the top of the head to the base of its spine using a stereomicroscopy. The life-history traits were integrated in the population growth rate (or intrinsic rate of natural increase) on days 10 and 21. Values were calculated using a fully-age classified model according to the Euler equation (1) (Meyer et al, 1986).

$$\sum_{x=0}^{x} l_x \cdot m_x \cdot e^{-r \cdot x} = 1 \qquad (1)$$

Where r = per capita rate of increase for the population (number per day), x = age of class (days; 1, 2, 3…a), a= oldest age class in the population (10 and 21 days in the present study), l_x = probability of surviving at age x, and m_x = fecundity at age x. Since this calculation involves a summation over several age classes, r cannot be isolated on one side of the equation to provide a closed-form algebraic solution. Instead, interactive calculations must be performed in order to determine an r value that satisfies Eq. 1.

3.2.3- Demographic analyses

The simply two-stages model (Levin et al. 1996) was used to calculate: 1- The contributions of the individual-level traits to the inhibition of the population growth rate following a continuous and pulse exposure of fenvalerate; 2- The sensitivity of the population growth rate to changes in individual-level traits (i.e. elasticities).

Contributions of the individual-level traits to the inhibition of the population growth rate

The decomposition analysis was performed in order to determine how much the effects of a specific treatment on each life-history trait contribute to the changes observed in the population growth rate (Caswell, 2000). For this, proportional contributions of individual-level traits (i.e. age at first reproduction, fecundity, juvenile survival, and adult survival) to population growth rate were defined. This is possible because the effects of fenvalerate treatment on population growth rate, measured relative to the control, can be decompose into contributions terms from each life-history following Levin et al. (1996: Eq. 16) and using the sensitivity equations given by Sibly et al. (2000: Eqs. 4–7). Population growth rate (r) calculated by Euler equation was converted to the population multiplication rate ($\lambda = e^r$) to use the sensitivity equations.

The elasticity analysis was performed to examine the relative sensitivity of population growth rate to changes in life-history traits under different exposure levels (Hansen et al., 1999, and Forbes et al., 2001). Elasticities analysis provides valuable information on how population growth rate will respond to changes in each individual life-history trait contributing to it. Elasticity is defined as the proportional changes of the population growth rate in response to changes in a life-history trait. It is given mathematically in terms of partial derivatives; thus, $(x_i/\lambda)(\partial\lambda/\partial x_i)$ indicates the elasticity (or relative sensitivity) of λ to x_i where $\partial\lambda/\partial x_i$ is the sensitivity of λ to changes in the individual-level trait, x_i. Determining whether population growth is more or less sensitive to toxicant stress than the most sensitive individual life-history trait is of great interest in order to predict how results from toxicity tests using individual-level endpoints are related to effects on population-level (Forbes et al., 2001).

3.2.4- Statistical analysis

The data were tested for normality (Kolmogorov-Smirnov test, $p < 0.05$) and homogeneity of variances (Levene's test, $p < 0.05$). When these requirements were not met, rank transformation was chosen (Potvin and Roff, 1993). The influence of the exposure duration on fenvalerate toxicity to *D. magna* was evaluated by applying two-ways analysis of variance (ANOVA) for the mean age at first reproduction, brood number and brood size. Application of the Jackknife procedure (Meyer et al., 1986) for estimates of population growth rate ($r = \log_e \lambda$) resulted in pseudovalues, which were analyzed by two-way ANOVA and Dunnett's test (Kammenga et al., 1996). Due to the complete mortality in the continuous level, the concentration level of 0.6 µg/L was excluded for the two-way analysis of variance in both exposure regimes. Repeated measures analysis of variance ($p < 0.05$) was applied in order to evaluate the progression of the concentration-response over time. Empty cells due to mortality were filled with the mean value of the level in order to avoid unbalance design. The fenvalerate treatments were compared with controls by the Dunnett test ($p < 0.05$).

3.3- RESULTS

Survival responses differed between both exposure regimes (Fig. 1). Significant mortality occurred at 0.1 and 0.3 µg/L with continuous and pulse exposure, respectively. Meanwhile, mortality was complete at 0.6 with the continuous exposure and at 3.2 µ/L with the pulse exposure (Fig. 1). Individuals die more rapidly with continuous regime. Mortality occurred within the first 4 days with continuous regime, and within the first 6 days with the pulse exposure (Fig. 1).

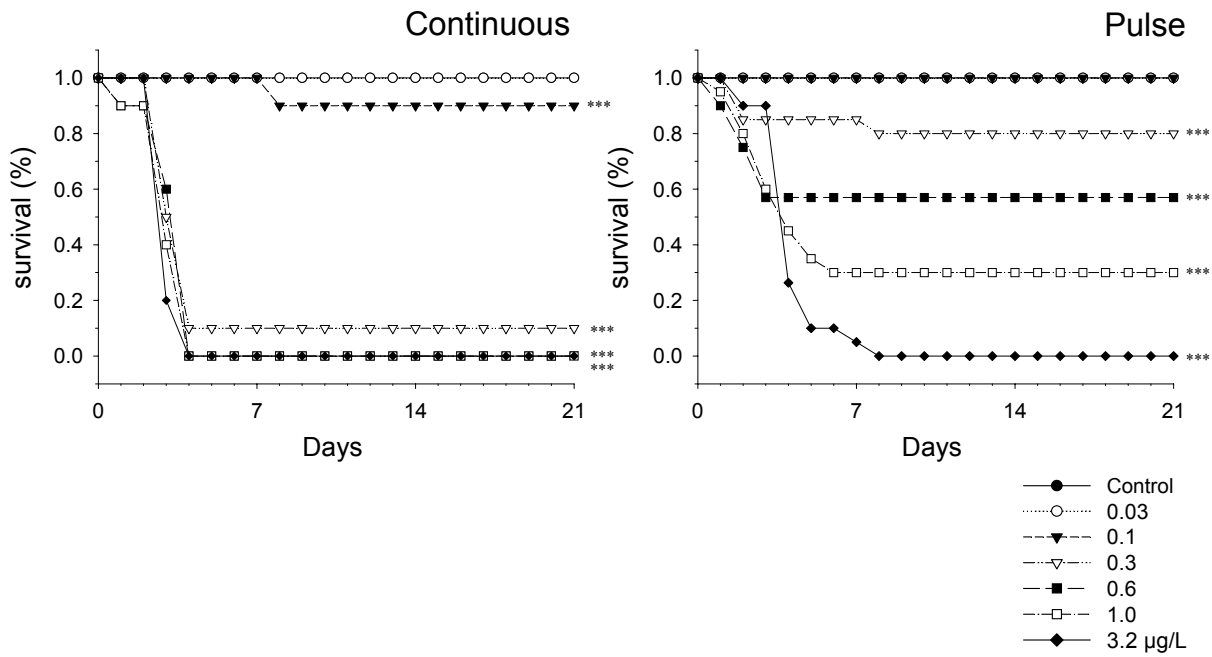


Fig. 1. Effects of continuous and pulse exposure to fenvalerate on the survival as percentage of initial number of individuals. Asterisks indicate significant (Dunnett's test: ***$p < 0.001$) differences from control treatments.

Exposure to fenvalerate also reduced the size of individuals on day 7 (Fig. 2), and increased significantly age at first reproduction at 0.1 and 0.3 µg/L under continuous and pulse exposure regime, respectively (Fig. 3).

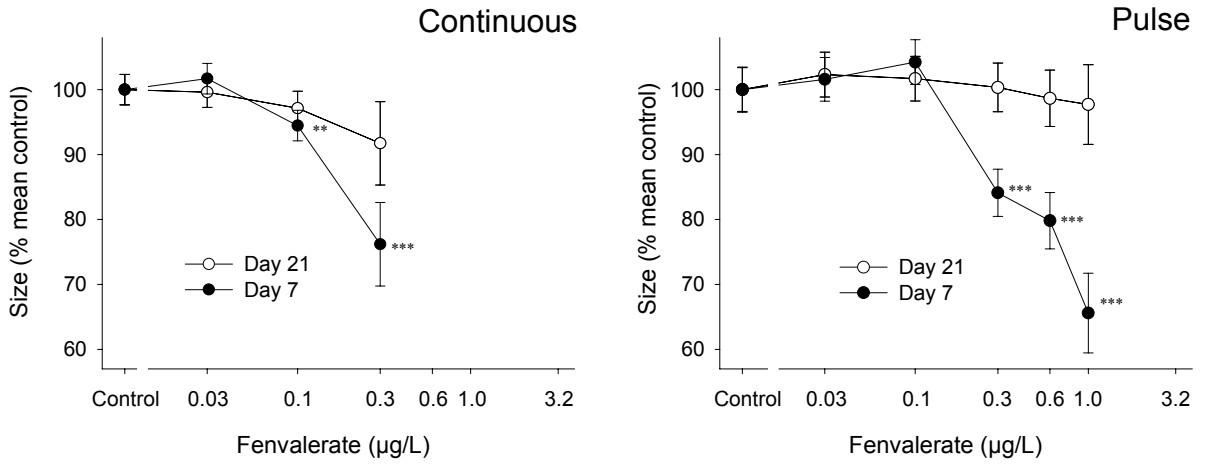


Fig. 2. Effects of continuous and pulse exposure to fenvalerate on the size of individuals on day 7 and day 21 (as % mean size of individuals in control treatments). Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett's test: **$p < 0.01$; ***$p < 0.001$) differences from control treatments.

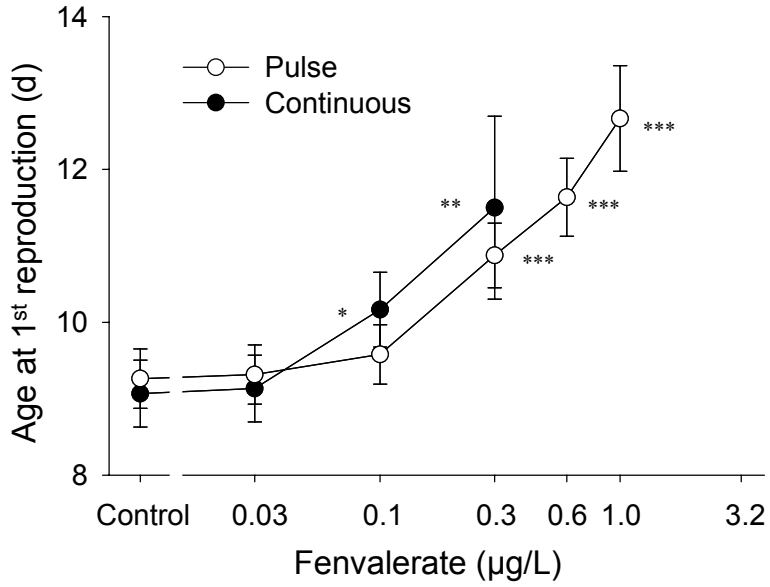


Fig. 3. Effects of continuous and pulse exposure to fenvalerate on the age at first reproduction. Error bars indicate 95% confidence limit. Asterisks indicate significant (Two-way ANOVA, Dunnett's test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

The relationship between size on day 7 and age to first reproduction was lineal with a similar slope for both exposure regimes (Fig.4.A). The number of broods per female was reduced by fenvalerate contamination (Table 1), being highly correlated with the age at first reproduction also with a similar slope for both exposure regimes (Fig. 4.B). In contrast, the brood size was significantly increased at 0.03 µg/L in the continuous exposure and at 1µg/L in the pulse exposure. On day 10, offspring per female was severely inhibited in both exposure regimes (Fig. 5).

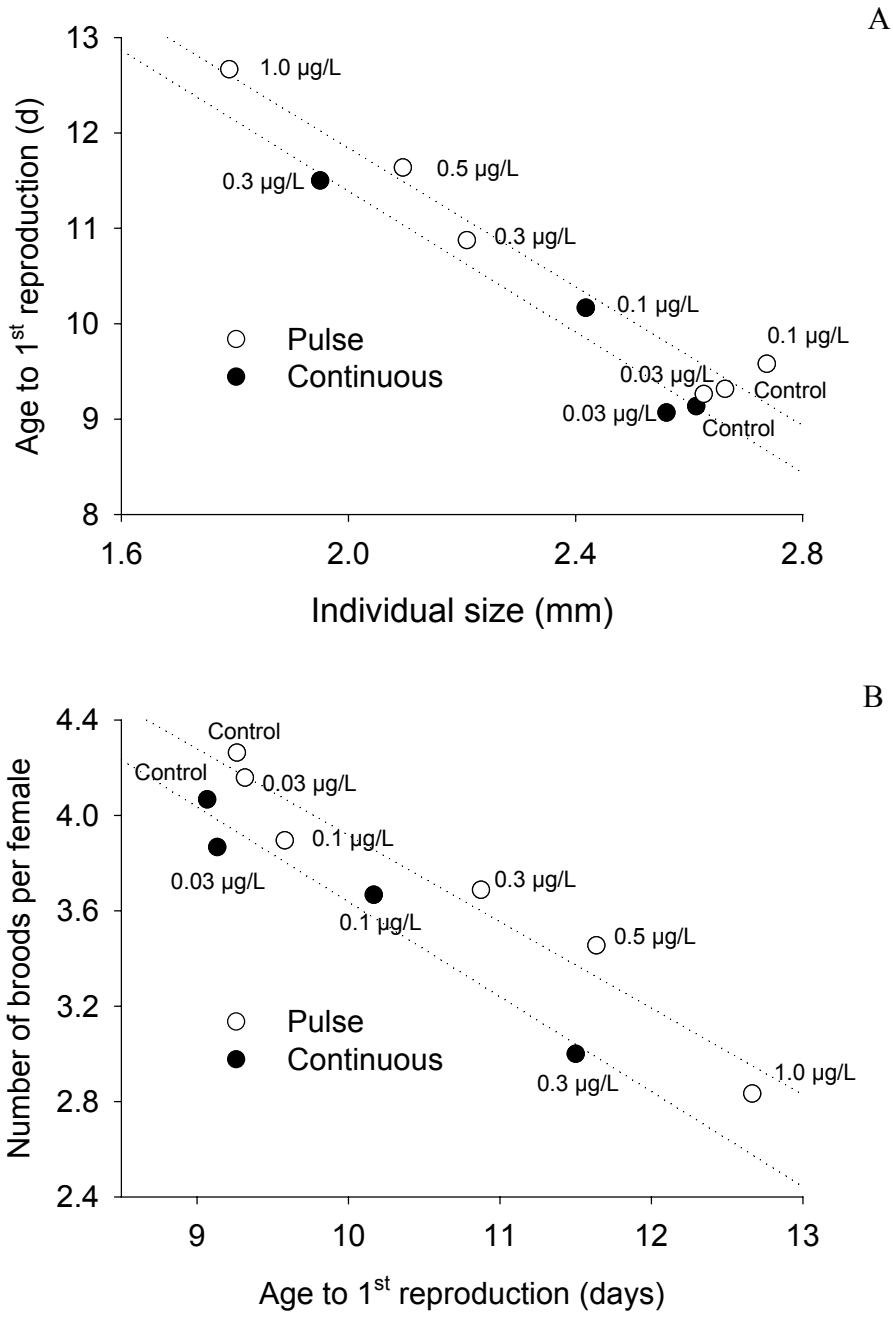


Fig. 4. A. Relationship between size on day 7, as carapace length (mm), and age at first reproduction (d), correlation for continuous (r^2 = 0.96, P< 0.004) and pulse (r^2 = 0.95, P< 0.01) regime. **B**. Relationship between age to first reproduction (d) and the number of broods per female for continuous (r^2 = 0.96, P< 0.02) and pulse (r^2 = 0.95, P< 0.001) regime.

Table 1. Effects of continuous and pulse exposure to fenvalerate (FV) exposures on number of broods per living female and brood size (number of offspring per brood) after 21 days. Mean ± 95% confidence limit.

Treatment	FV (µg/L)	Number of broods	Brood size
Continuous	Control	4.07±0.25	6.03±0.38
	0.03	3.87±0.25	6.66±0.3*
	0.1	3.67±0.28**	5.91±0.42
	0.3	3.00±0.68***	5.66±1.04
	0.6	-[a]	-[a]
	1	-[a]	-[a]
Pulse	Control	4.26±0.22	5.49±0.34
	0.03	4.16±0.22	5.77±0.34
	0.1	3.86±0.22	5.73±0.34
	0.3	3.69±0.24**	5.78±0.37
	0.6	3.45±0.29***	5.73± 0.44
	1.0	2.83±0.39***	6.97±0.60**

No values were calculated due to [a] complete mortality. Asterisks indicate significant (Two-way ANOVA, Dunnett's test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences to control treatments.

After 21 days, the absence of significant effects indicated a recovery of individual size and offspring per female in both exposure regimes (Fig.2 and Fig.5). Population growth rate was also severely inhibited at the beginning of reproduction; however, it started to recover rapidly with cumulated offspring per female over time (i.e. with the progress of the reproduction) (Fig. 6). The extent of the recovery depended on the final number of offspring per female (Fig. 6).

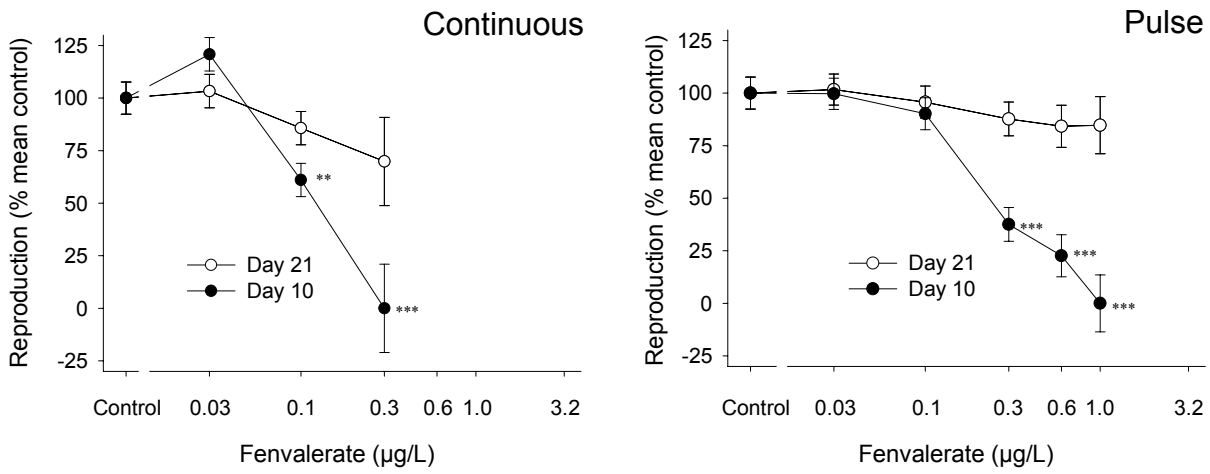


Fig. 5. Effects of continuous and pulse exposure to fenvalerate on reproduction as % of the mean of offspring per living female in control treatments on day 10 and 21. Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett's test: ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

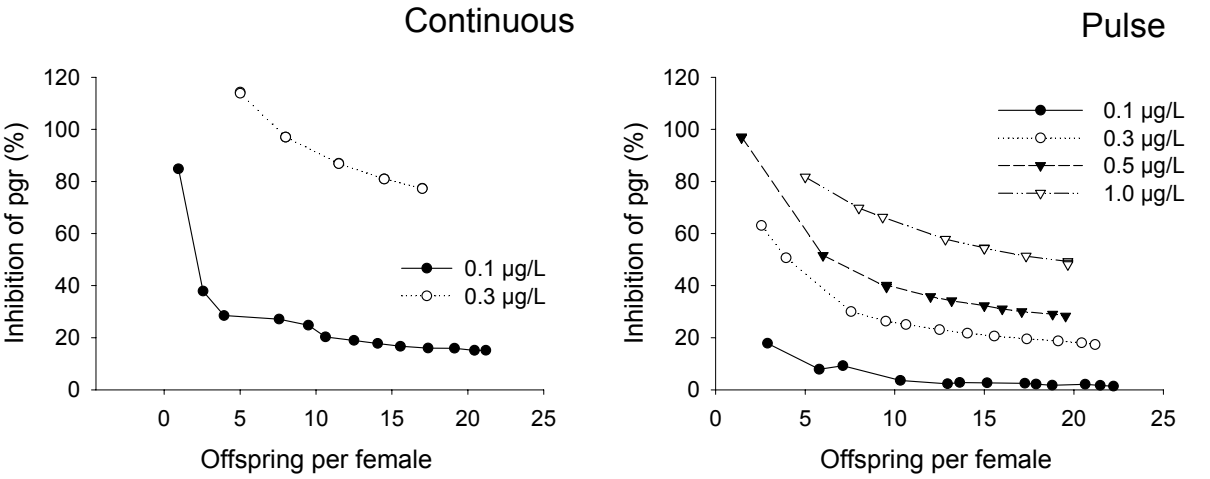


Fig. 6. Decrease in the inhibition of population growth rate (pgr) with increasing accumulative neonates per female. No values were calculated at 0.6 and 1 µg/L for the continuous exposure due to complete mortality.

Although recovery was substantial when comparing values on day 10 and day 21, population growth rate on day 21 remained affected at concentration as low as 0.1 and 0.3 µg/L under continuous and pulse exposure, respectively (Table 2). Population growth rate on day 21 was highly correlated with mortality in both exposure regimes (Fig. 7) with a steeper slope was for the continuous exposure. The Jackknife estimates showed that exposure duration influenced responses of population growth rate to fenvalerate on day 21 (Two-way ANOVA, $p<0.05$).

Table 2. Effects of pulse and continuous exposure to fenvalerate on population growth rate (d^{-1}). Mean ± 95% confidence limit.

Fenvalerate	Continuous exposure		Pulse exposure	
(µg/L)	Day 10	Day 21	Day 10	Day 21
Control	0.193±0.10	0.235±0.02	0.203±0.09	0.231±0.02
0.03	0.209±0.104	0.232±0.02	0.201± 0.09	0.228±0.02
0.1	0.119±0.104	0.199±0.02***	0.187±0.09	0.228±0.02
0.3	-[b]	0.054±0.04***	0.075± 0.09**	0.191±0.02***
0.6	–[a]	–[a]	0.006±0.100***	0.166±0.02***
1	–[a]	–[a]	-[b]	0.120±0.03***
3.2	–[a]	–[a]	–[a]	–[a]

No values were calculated due to [a] complete mortality or [b] marked delay of first reproduction. Asterisks indicate significant differences from control treatments. (Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

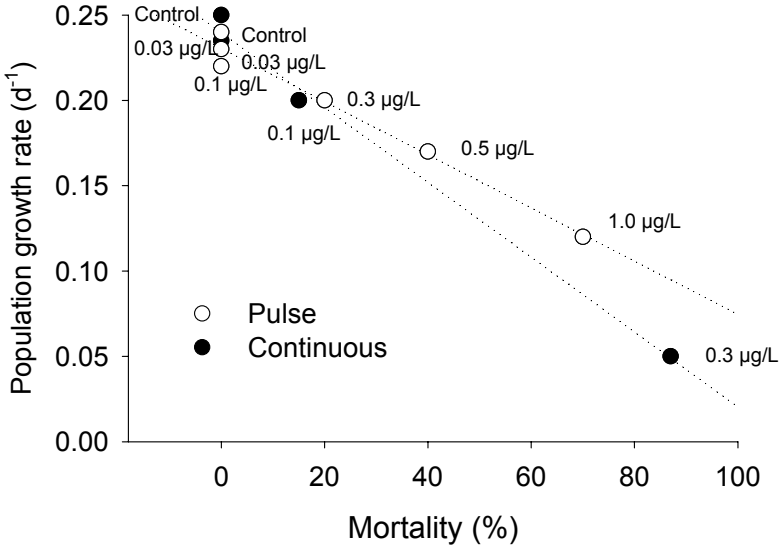


Fig.7. Relationship between survival, as percentage of initial individuals, and population growth rate (d^{-1}), correlation for continuous ($r^2 = 0.99$, $P < 0.002$) and pulse ($r^2 = 0.98$, $P < 0.001$) regime.

The decomposition analysis indicated that effects of fenvalerate on juvenile survival were the greatest contribution to the inhibition of the population growth rate (Fig. 8). It explained 96 and 87% of effects on population growth rate at highest concentration in the continuous (0.3 µg/L) and in the pulse regime (1 µg/L), respectively. Age at first reproduction explained 3 and 12% of effects on population growth rate at highest concentration in the continuous (0.3 µg/L) and in the pulse regime (1 µg/L), respectively. Reproductive output had a minor contribution to the inhibition of population growth rate, likely due to the recovery observed on day 21. No contribution from adult survival was registered because mortality did not occur beyond day 6 in either regime (Fig. 1).

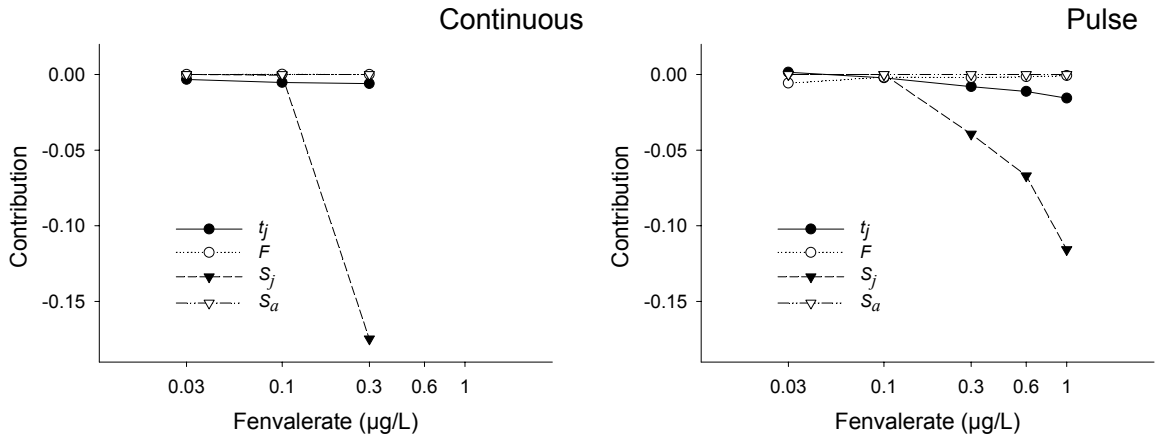


Fig.8. Contribution of individual-level traits to differences in population growth rate between control treatment and each fenvalerate treatments: age to first reproduction (t_j: days to first reproduction), reproductive output (F: offspring/female/day), juvenile survival (S_j: mean survival rate before first reproduction), and adult survival (S_a: mean survival rate after first reproduction).

The elasticity analysis under control conditions indicated that population growth rate was more sensitive to changes in juvenile survival than to changes in adult survival, reproductive output and age at first reproduction (Fig. 9) – the latter traits being ranked in order of importance. Under fenvalerate exposure, elasticity to juvenile survival increase and to age at first reproduction decreased with increasing fenvalerate concentration in both exposure regimes (Fig. 9). However, s steeper slope was observed for the continuous exposure

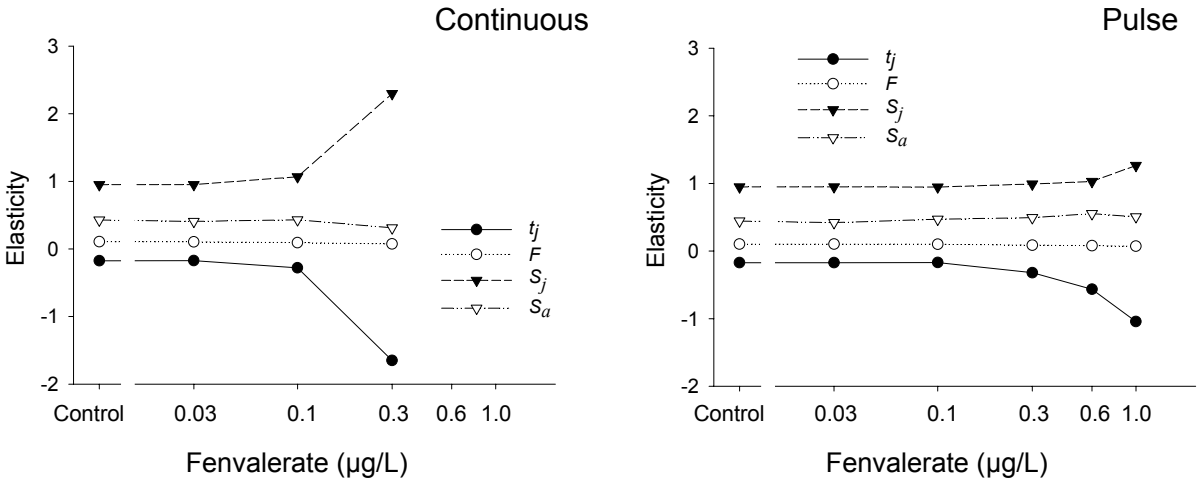


Fig.9. Sensitivity of population growth rate to changes in individual-level traits (elasticity) plotted against fenvalerate concentrations: age to first reproduction (t_j: days to first reproduction), reproductive output (F: offspring/ female/day), juvenile survival (S_j: mean survival rate before first reproduction), and adult survival (S_a: mean survival rate after first reproduction).

3.4- DISCUSSION

3.4. 1- Effects on individual-level traits and on population growth rate.

Exposure duration influenced survival responses to fenvalerate on day 21 (Two-way ANOVA, $p < 0.05$). Differences between continuous and pulse exposure regimes increased with fenvalerate concentration. Complete mortality (i.e. 100% mortality) in the continuous regime occurred at more than five times lower concentration than in the pulse regime. As a result, sublethal responses occurred in a broad range of concentration with the pulse regime. The first-observed sublethal response was a retardation (but not an inhibition) of somatic growth in both exposure regimes - small animals on day 7 recovered on day 21 (Fig. 2). Next observation was a delay in development (i.e. delay in reaching first reproduction.) (Fig. 3). Similar responses were observed for the caddisfly *Limnephilus lunatus* when exposed to a 1-h pulse of fenvalerate (Liess and Schulz, 1996). Growth retardation most likely was the primary cause of developmental delay since both were highly correlated (Fig. 4 B).

In addition, the delay in development was correlated to the number of broods per female with a similar slope in both exposure regimes (Fig. 4 B). The reduction of broods produced a severe inhibition of the reproductive output on day 10, but the initial losses were buffered on day 21 as females progressed with their reproduction in both exposure regimes (Fig. 5 and Fig. 6). The correlations showed by figure four suggesting that most likely the observed growth

retardation induced a decrease of the population growth rate by causing a delay in development. In a previous study, somatic growth and population growth rate were linearly correlated under varying natural stress conditions (temperature and food, among others) (Lampert and Trubetskova, 1996); however, this correlation was not found when *Daphnia* was exposed to DCA, a toxicant that directly affects reproduction (Trubetskova and Lampert, 1996). In contrast, this study uses fenvalerate, which affects reproduction indirectly through growth retardation and the subsequent delay in development. It is crucial to notice that effects on reproduction were primarily caused by delay in development since brood size was not decreased by fenvalerate at any concentration level (Table 1) – in contrast to a toxicant like DCA, which would affect brood size. That is why population growth rate started to recover according to offspring per female (Fig. 6), though clearly this recovery was restricted by the mortality occurred at the beginning of the experiment.

3.4. 2- Demographic analysis

In the decomposition analysis, by definition, contributions in control treatments are zero and thus contributions to decrease of population growth rate are negative (Barata et al, 2002). The analysis sowed that the most sensitive endpoint (i.e. survival) was the major contribution to the inhibition of the population growth rate (Fig. 8). It is important to note that mortality only occurred before maturity (i.e. decrease of juvenile survival). Although these contributions increased in both exposure regimes with increasing fenvalerate concentration, the slope was steeper in the continuous exposure where mortality was more severe (Fig. 8). On the other hand, the low contribution of reproductive output and age at first reproduction rate may reflect the recovery observed in reproduction on day 21 (Fig. 5). Age at first reproduction was more relevant in the pulse regime than in the continuous regime where survival was more severely affected. The impact of age at first reproduction on population-level seems to be more relevant in semelparous, such as the caddisfly *Limnephilus lunatus* (Schulz and Liess, 2000), most likely because the buffering mechanism proposed above cannot take place these species.

Elasticity is defined as the sensitivity of population growth rate to changes in individual-level traits (Forbes et al., 2001). Under control conditions, population growth rate was more sensitive to changes in juvenile survival than to age at first reproduction. The low elasticity to fecundity may explain why no significant positive effects on population growth rate were observed although significant stimulatory effects of fenvalerate on brood size were registered in

both exposure regimes (Table 1). Similar results were observed with the polychaete *Capitella* sp. I exposed to sediment-associated 4-n-nonylphenol (Hansen et al., 1999). By having distinct impacts on the sensitivity of the population growth rate to changes in individual life-history traits, environmental fluctuations can have important effects on the direction of life-history evolution (Kammenga et al, 2001). In this way, the relevance of elasticity to juvenile survival seems to be in accordance with the hypothesis that the evolution of increased iteropatity in *Daphnia* species responds to low reproductive success due to competition and predation (Schwartz, 1984). It is interesting to note that fenvalerate exposure increased the elasticity of population growth rate to survival in both exposure regimes but in a large extent in the continuous exposure after 0.1µg/L (Fig. 9).

3.4.3- Comparison between exposure duration influence under food restrictive and normal food conditions.

As was established earlier, food restrictive conditions in this study are defined as 1/3 of normal-food conditions, which is the one required to obtain at least 60 neonates per female in control treatments (OCDE, 1997). When comparing the influence of exposure duration on *Daphnia magna* under high and low food conditions, survival responses were more severely affected under the latter (chapter II). Nonetheless, fenvalerate under normal-food affected age at first reproduction in a similar proportion to the one observed in the present study. However, the population growth rate in the pulse regime under normal-food recovered up to reaching values that were almost the same as those in control treatments (chapter II); under low-food conditions, instead, population growth rate reached 48% of control values even on day 21. This likely occurred because of two reasons: 1) as mentioned above, mortality was more severe under low-food; and 2) under low-food females released less broods making difficult to buffer the impact of the initial losses. In agreement with 1), the contribution of effects on survival to the decrease of population growth rate was smaller under normal-food thus increasing the relevance of the contribution of the delay in age at first reproduction (chapter II). Elasticity analysis showed that under control conditions, sensitivity of population growth rate to changes in survival was similar in both food conditions (normal food data are not shown). However, fenvalerate increased more elasticities of population growth rate to survival under normal-food conditions and decreased less elasticity to age at fist reproduction (data not shown). This results confirm the suggestion that population growth rate becomes insensitive to changes in individual-level traits when approaches to steady state ($r = 0$, or $\lambda = 1$) (Forbes et al, 2001).

3.5- CONCLUSIONS

The low-food conditions increase the differences in survival between exposure regimes. The more severe mortality limited the recovery of the population growth rate. Both exposure regimes, the population growth rate was equally responsive than survival (the most sensitive individual life-history traits). In terms of effect strength, however, the effects of fenvalerate on survival were attenuated by the population growth rate.

Chapter IV

Feeding responses: a link between individual to population levels responses of *Daphnia magna* Straus following short-term (24-h) exposure to the pyrethroid fenvalerate.

Abstract: The aim of the present experimental study is to establish a relationship between fenvalerate-induced changes in the feeding responses of *D. magna* and the subsequent population-level effects and recovery. By using ^{15}N-tracer incorporation, ^{15}N-tracer turnover over time, and filtering experiments, the feeding responses were investigated and compared with results of Life table response experiment. The comparison of the results of these independent experiments shows clearly that a short-term (24h) exposure to fenvalerate caused a transient inhibition of *D. magna* feeding responses. Through growth retardation, this can delay the development with the subsequent inhibition and recovery of the reproductive output and population growth. It is worth to notice that inhibition and recovery of the population growth rate and feeding responses occurred at the same concentrations (≥ 0.3 µg/L). This indicates the utility of the feeding responses to understand and predict the impact of stressors at high organizational levels.

Key words: Pulse exposure, feeding inhibition, pyrethroids, *Daphnia magna,* and Stable-isotope ^{15}N.

4.1- INTRODUCTION

The changes in the feeding behaviour of zooplankton organisms may be the first effects of environmental perturbation (Day et al 1987). Day and Kaushik (1987b) found that 24h-pulse of fenvalerate caused a reduction in the algae filtration and assimilation of three zooplankton species, and thus the authors suggested that the impairment may help to explain observed changes in growth, which could lead to a reduce chance of reproduction. On the other hand, a short-term exposure to fenvalerate caused a delay in the development of the caddisfly *Limnephilus lunatus* (Liess and Schulz, 1996). For *Daphnia magna*, several studies have demonstrated that toxic impairment of feeding rates at the individual level has direct effects on population parameters such as growth and reproduction (Baird et al., 1990; Bradley et al, 1991;

Barata and Baird 2000). In this way, Maltby (1999) proposed that the effects of stressors on individual energy budget can predict effects on populations. Furthermore, extrapolation from short-term effects on individual-level to long-term effects on populations can be achieved by comparing the sensitivities of demographic responses with those observed in individual life-history traits (Forbes and Calow, 1999). Therefore, the objective of this study was to investigate whether feeding responses to fenvalerate could be used to explain and predict population-level responses of *Daphnia magna* (Fig.1).

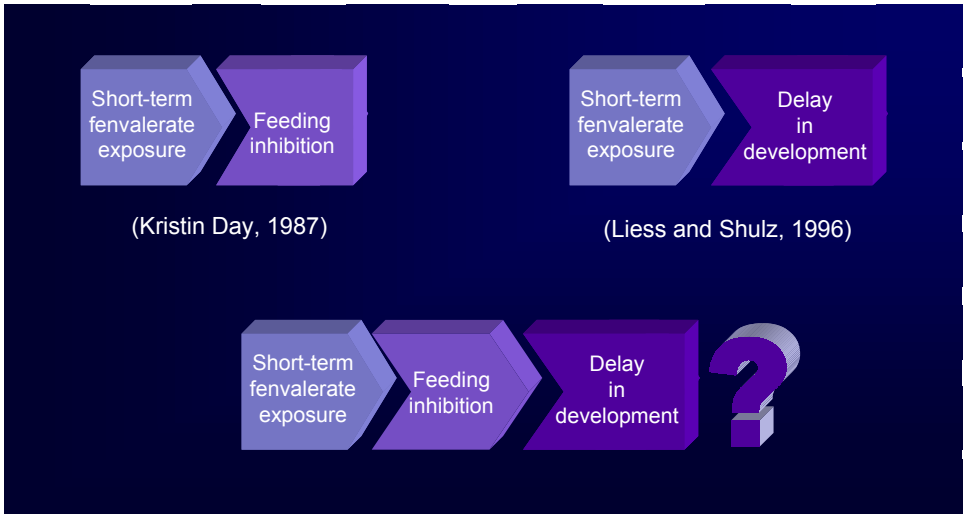


Fig. 1. Possible relationship between fenvalerate effects on feeding inhibition and effects on life-history trait such as age at first reproduction (i.e. age at development).

4.2- MATERIALS AND METHODS

4.2.1- ^{15}N-tracer experiments

The stable-isotope ^{15}N was used to study the effects of short-term fenvalerate exposure on feeding responses of *D magna* of individuals (Fig. 2). The incorporation process was investigated in two independent experiments: 1) ^{15}N incorporation experiment, where neonates (<24-h-old) of *Daphnia magna* were exposed to a short-term exposure (24h) of fenvalerate (0, 0.1, 0.3 and 0.6 µg/L), and transferred into fresh M7 medium containing ^{15}N-labeled algae as unique food recourses. The microalgae *Desmodesmus subspicatus* was labeled growing it in Grimme and Boardmann (1972) medium containing KNO_3 (10% ^{15}N). The individual size and ^{15}N abundance [atom-%] were measured 5 day after exposure, Dead animals were removed and registered daily,

keeping the same feeding condition; and 2) ^{15}N turnover experiment where by feeding neonates with ^{15}N-labeled algae, ^{15}N-labeled mothers were obtained. The offspring of these mothers (^{15}N-labeled neonates) were exposed to 24h-pulse of fenvalerate (0 and 0.6 µg/L), and fed with non-labeled algae. The individual size and ^{15}N abundance [atom-%] were measured at day 0, 1, and 6 after exposure. The algae were labeled in the same way described above but with medium containing KNO_3 (20% ^{15}N). Dead animals were removed and registered daily, keeping the same condition among fenvalerate treatments. The size was measured from the top of the head to the base of its spine using stereomicroscopy. The ^{15}N abundance [atom-%] was measure using ^{15}N analyzer system NOI 7 (Fischer Instrument Analyzer, Leipzig, Germany).

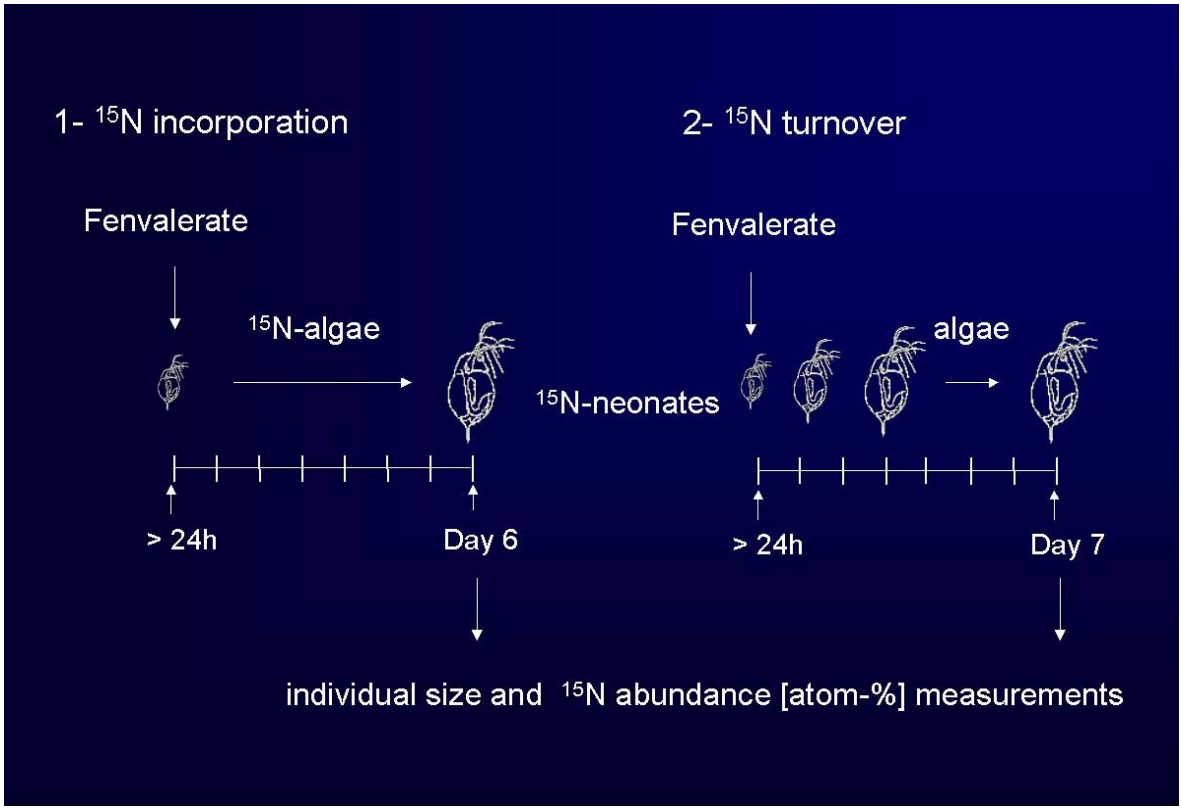


Fig. 2. ^{15}N-tracer technique applied to identify the effects of fenvalerate on growth and feeding behavior of Daphnia magna.

4.2.2- Filtering rate experiment

Neonates (<24-h-old) were exposed to a 24-h-pulse of fenvalerate (0, 0.3, 0.6, and 1 µg/L), and then animals in groups of 8 individuals were transferred to M7 medium. Filtering rates (cells/individual/h) were determined during exposure and on 1, 2 and 3 days after exposure as there is a change in cell density during 24 h according to the method described by Barata and Baird (2000). Cell density was measured using an electronic particle counter with a 16-µm-orifice tube (Casy, Stuttgart, Germany). Four replicas per fenvalerate concentration and three blank replicas (medium and food with out animals) were established. Dead animals were removed and registered daily, keeping the same condition described for *D. magna* culture among fenvalerate concentrations. The size (mm) of 10 randomly selected individual per fenvalerate concentration was measured animals as described above on day 4, and 10 after exposure.

4.2.3- Life table response experiments

Neonates (<24-h-old) from 3-week-old females, were exposed to 0, 0.1, 0.3 0.6 and 1 µg/L of fenvalerate in 24-h-pulse. Alter exposure, 15 neonates per concentration were individually placed in 80 ml of Elendt-M7 medium, fed batch-cultured green microalgae (*Desmodesmus subspicatus* ~ 0.07 mg C/*Daphnia*/day), and maintained for 14 days at 20°C ± 1°C in a light-dark regime of 16:8h, with light intensity of ~15 µmol/m^2/s (OECD, 1998). Food and medium were renewed three times a week. Every day, dead and newborn animals were removed and counted. Age at first reproduction was determined from the number of produced neonates recorded daily. The individual size was measured on day 7 after exposure from the top of the head to the base of its spine using a stereomicroscopy. The life-history traits were integrated in the population growth rate (intrinsic rate of natural increase). Values were calculated according to the Euler equation (1)

$$\sum_{x=0}^{x} l_x \cdot m_x \cdot e^{-r \cdot x} = 1 \qquad (1)$$

Where r = per capita rate of increase for the population (number per day), x = age of class (days; 1, 2, 3…a), a= oldest age class in the population (day 14 in the present study), l_x = probability of surviving at age x, and m_x = fecundity at age x. Since this calculation involves a summation over

several age classes, r cannot be isolated on one side of the equation to provide a closed-form algebraic solution. Instead, interactive calculations must be performed in order to determine an r value that satisfies Eq. 1.

4.2.4- Fenvalerate exposure and measurement

Fenvalerate, (RS)-α-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate (CAS: 51630-58-1), was obtained from Riedel-de Haën®, Seelze, Germany (99.9%). The carrier solvent DiMethylSulfOxide (DMSO, Merck®, Darmstadt, Germany, 99.8%) was used to add fenvalerate to the test medium. The maximum amount of DMSO was 0.00003% (v/v). In all experiments, animals fed during exposures in both regimes, the alga suspension (~0.45 mgC/L) being added to fenvalerate solutions before the *Daphnia* individuals were introduced. Actual exposure concentrations were only determined for 0.6 and 1 µg/L test solutions (n = 3) at t = 1h to match detection limits. Additionally, a 1 µg/L test solution (n = 3) was measured at t = 24h. Samples were measured in the Institute for Ecological Chemistry and Waste Analysis, Technical University of Braunschweig, Germany. Solid-phase extraction of 1-L volumes was carried out using C18-columns (Baker, Phillipsburg, NJ, USA). The analyses were performed by means of gas chromatography/mass spectormetry applying electron impact ionisation and selected ion monitoring mode (Agilent 6890 Series GC System with Agilent 7683 Series Injector and Agilent Network Mass Selective Detector; all Agilent, Waldbronn, Germany). Analytical measurements of the 0.6 and 1 µg/L test solutions at t = 1h showed a reduction in the nominal concentrations of 50-60% (0.2 ±0, 0.43 ±0.12, and 1.47 ± 0.28 µg/, respectively). The actual concentration of the 1.0 µg/L test solution decreased to 0.37±0.06 µg/L after 24h. Nominal concentrations are given in the following sections.

4.2.5-Statistical analysis

The data were tested for normality (Kolmogorov-Smirnov test, $p < 0.05$) and homogeneity of variances (Levene's test, $p < 0.05$). When these requirements were not met, rank transformation was chosen (Potvin and Roff, 1993). Repeated measures analysis of variance ($p < 0.05$) was applied in order to evaluate the progression of the concentration-response over time. Empty cells due to mortality were filled with the mean value of the level in order to avoid unbalance design. The fenvalerate treatments were compared with controls by the Dunnett test (p

< 0.05). Jackknife estimates (Meyer et al. 1986) for survival outcomes and population growth rate were calculated and then Dunnett's test ($p < 0.05$) was applied (Kammenga et al, 1996).

4.3- RESULTS

4.3.1- ^{15}N-tracer incorporation experiment

Five days after the 24-h-pulse exposure to fenvalerate, *D. magna* individuals fed with ^{15}N-labeled algae exhibited a reduction in ^{15}N abundance [atom-%] and in size at concentrations ≥ 0.3 µg/L (Fig. 3).

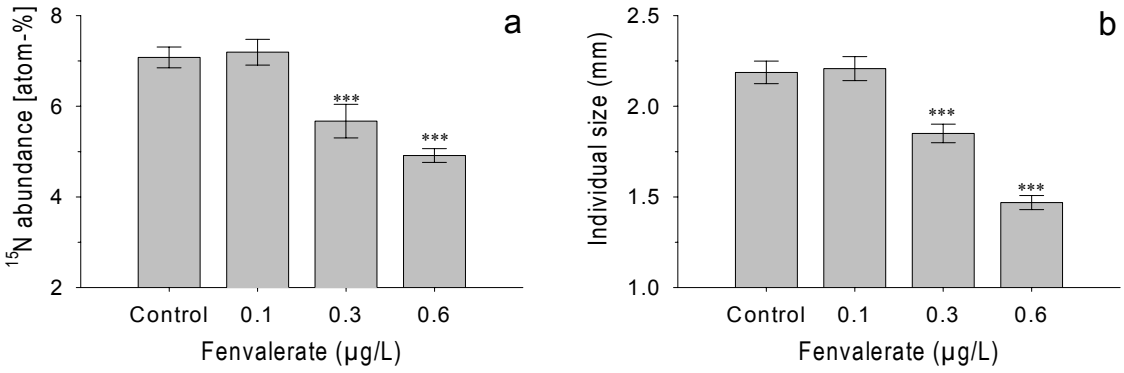


Fig. 3. Effects of 24-h-pulse exposure to fenvalerate on feeding responses measured as: a) the increase of ^{15}N abundance [atom-%]; and b) the size (mm) of exposed and control individuals. Error bars indicate 95% confidence limit. Asterisks indicate significant (Dunnett's test: ***$p < 0.001$) differences from control treatments.

4.3.2- ^{15}N-tracer turnover experiment

The bodies of ^{15}N-labeled neonates exposed for 24-h to 0.6 µg/L and fed with non-labeled algae showed no significant reduction of ^{15}N abundance [atom-%] from day 0 to day 1. Simultaneously, no significant growth change in the size of these individuals was observed (Fig. 4). In contrast, individuals in the control treatments also fed with non-labeled algae exhibited a significant growth and reduction of ^{15}N abundance [atom-%] after the same period. On day 6, however, exposed and control animals reached similar size and ^{15}N abundance [atom-%] indicating that animals recovered between day 1 and day 6 (Fig. 4).

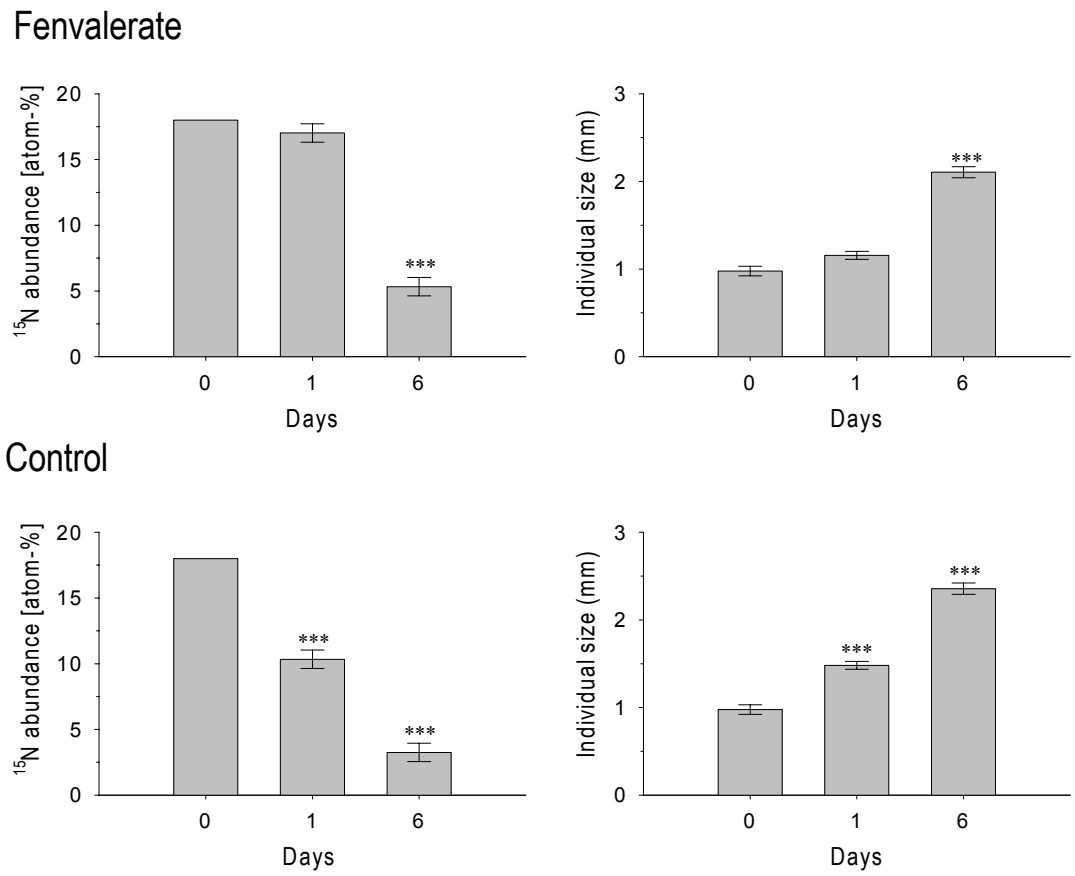


Fig. 4. Feeding responses measured as the decrease of ^{15}N abundance [atom-%] in the body and the size (mm) of ^{15}N-labeled animals fed with non-labeled algae. Feeding responses of animals exposed to 24-h-pulse exposure of fenvalerate (0.6 µg/L) and kept under control conditions are compared. Error bars indicate 95% confidence limit. Asterisks indicate significant (Dunnett's test: ***$p < 0.001$) differences from control treatments.

4.3.3- Filtering rate experiment

Filtering rates (10^6. filtered cells.ind^{-1}.h^{-1}) were significantly reduced at concentration ≥ 0.3 µg/L during exposure. However, they started to recovery 1 day after exposure, significant reduction was only observed at 1 µg/L. No significant effects were observed 2 and 3 days after exposure indicating that recovery occurred (Fig. 5). The 24-h- pulse exposure to fenvalerate also resulted in a significant reduction in the size of individuals that was registered 4 days after exposure. However, no significant effects were observed 10 days after exposure (Fig. 6).

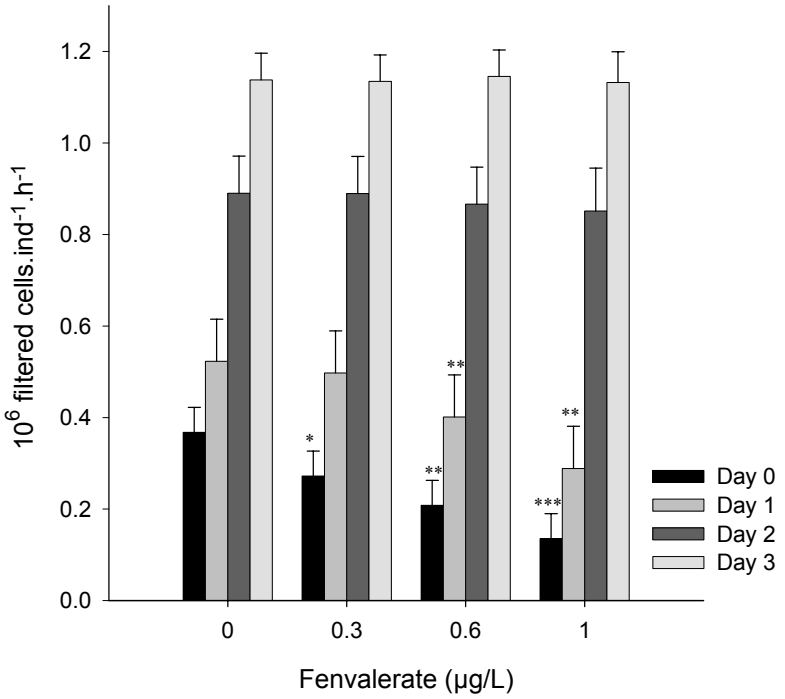


Fig. 5. Effects of 24-h-pulse exposure to fenvalerate on filtering rates measured as filtered cells per individual per hour on 0, 1, 2 and 3 days after exposure. Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett's test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

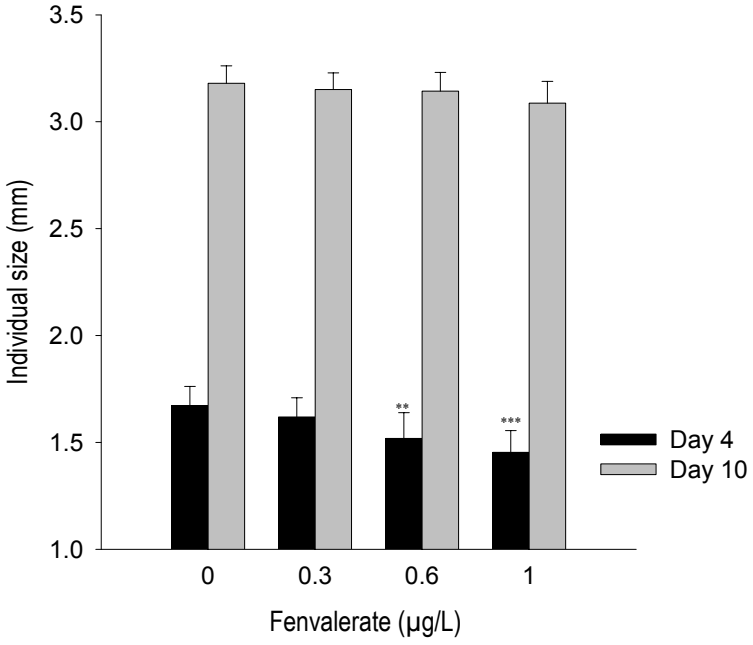


Fig. 6. Effects of 24-h-pulse exposure to fenvalerate on individual size (mm) measured on day 4 and day 10 after exposure Error bars indicate 95% confidence limit. Asterisks indicate significant (Repeated measures analysis of variance, Dunnett's test: ** $p < 0.01$; *** $p < 0.001$) differences from control treatments.

4.3.4- Life-table response experiments

The results shown in Table 1 indicate that survival and individual size were affected at concentration ≥ 0.6 and 1 µg/L, respectively. Age at first reproduction and offspring per female were affected at concentrations ≥ 0.6 µg/L. Population growth rate was affected at concentration ≥ 0.3 µg/L. The mean age at first reproduction was highly correlated with the mean individual size and the mean offspring per female (i.e. neonates per mother) (Fig. 7). The inhibition of population growth rate decreased increasing of offspring per female over time (Fig. 8). The relationship between individual size, age at first reproduction and offspring per female showed by the correlations; as well as, the decrease of the inhibition of the population growth rate were previously observed in chapter III.

Table 1. Effects of 24-h-pulse of fenvalerate on survival (% of initial animals), individual size (mm), age at first reproduction, offspring per female and population growth (d^{-1}) on day 14 after exposure. Mean ± 95% confidence limit.

	Fenvalerate (µg/L)				
	Control	0.1	0.3	0.6	1
Survival	100±1.92	100±1.92	100±1.92	80±1.92***	66±1.92***
Individual size	3.02±0.25	2.92±0.25	2.74±0.25	2.62±0.28	2.41±0.26**
Age at first reproduction	9.2±0.4	9.3±0.4	10.0±0.4	11.6±0.5***	12.6±0.7***
Offspring per female	34.8±4.4	32.1± 4.2	28.7±4.2	23.3±4.6***	20.4±5.0***
Population growth rate	0.32±0.02	0.3± 0.02	0.29±0.015*	0.25±0.02***	0.23±0.02***

Asterisks indicate significant differences from control treatments. (Dunnett test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

4.4- DISCUSSION

In the ^{15}N-tracer incorporation experiment, ^{15}N-labeled algae were the unique source of ^{15}N-tracer. Therefore, ^{15}N abundance [atom-%] measured in body of individuals was a result of food acquisition and assimilation. Thus, the significant lower ^{15}N abundance [atom-%] at ≥ 0.3 µg/L of fenvalerate is associated with an inhibition of these processes (Fig. 3a). Animals with low ^{15}N abundance [atom-%] were at the same time significant smaller compare to those in control treatments confirming that fenvalerate exposure inhibited feeding processes (i.e.

acquisition and/or assimilation of food was affected).

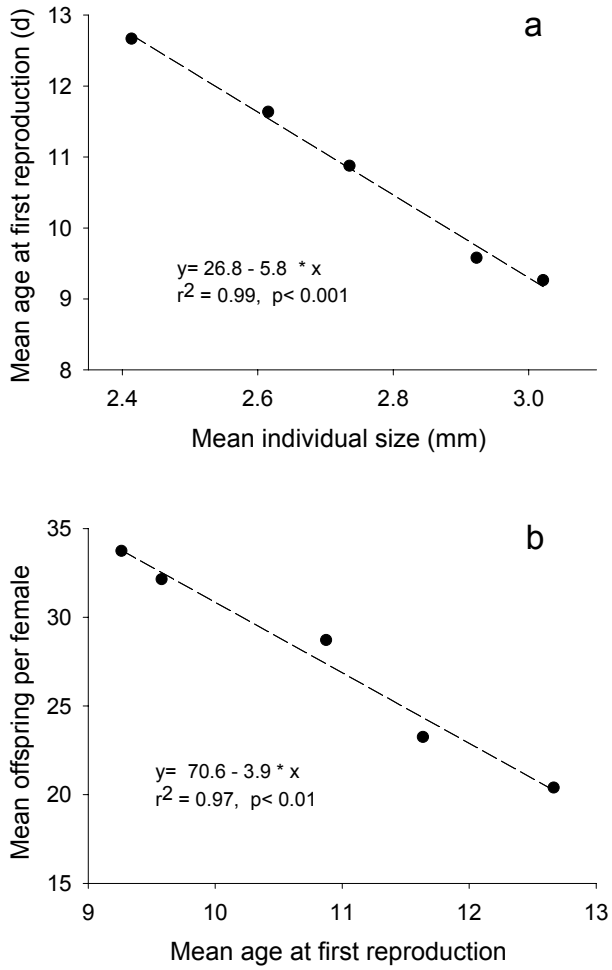


Fig. 7.a Relationship between the mean size of the individuals (mm) on day 7, and the mean age at first reproduction. **b.** Relationship between the mean age to first reproduction (d) and the mean number of offspring per female.

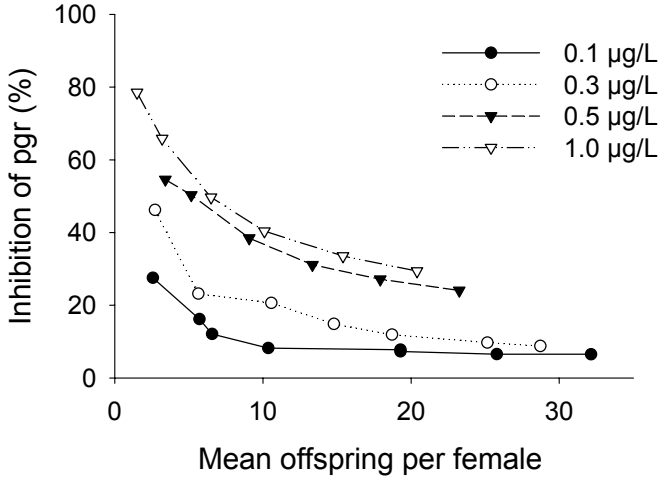


Fig. 8. Decrease in the inhibition of population growth rate (pgr) with increasing the mean number of offspring per female.

When exposed to fenvalerate, the postlarval shrimp *Palaemonetes pugio* had an elevated respiration rate suggesting an increase in the metabolic demand due to detoxification cost, that may result in less energy available for production of new tissue (Mckenney and Hamaker, 1984). This may explain the small size of the animals exposed to fenvalerate observed in the present studied. It is worth to notice, however, that detoxification cost cannot be considered as the exclusive cause of the smaller size exhibited by exposed animals, because no small animals with high ^{15}N abundance [atom-%] were observed (Fig. 3).

The ^{15}N turnover experiment, where ^{15}N abundance [atom-%] decreased with the assimilation of non-labeled algae, confirmed that short-term fenvalerate exposure affected ^{15}N abundance [atom-%], which was closely related with the decrease in individual size (Fig. 3). This experiment showed, as well, that effects due to fenvalerate exposure were only observed one day after exposure and recovery occurred within the week following exposure (Fig. 4). The filtering rate experiment confirms this finding, showing that two days after exposure no significant effects on filtering rate could be observed (Fig. 5). This experiment also indicates that the decrease in ^{15}N abundance [atom-%] observed in the ^{15}N-tracer experiment was most likely induced by fenvalerate effects on the filtering rate. Thus, it is possible to propose that animals exposed to $\geq$ 0.3 µg/L of fenvalerate did not assimilate ^{15}N in the same extent as the control individuals because they could not filtrate the algae in the same extent. Similar inhibition of filtering rate and recovery trend was observed when *D. magna* was exposed to the pyrethroid permethrin and λ-cyhalothrin (McWilliam and Baird, 2002).

The results of filtering experiment in the present study does not allow to discard possible effects of fenvalerate on nitrogen assimilation, similar than those reported by Mckenney et al. (1998), in the estuarine shrimp *P. pugio*. The authors analyzed carbon and nitrogen accumulation throughout larval development reporting also a growth alteration, but they did not measure filtering rate. Nevertheless, Day and Kaushik (1987b) suggested that since ingestion and filtration of food by filter-feeding zooplankton (i.e. *D. magna)* require coordinate movement of appendages, toxicants such as fenvalerate, which affects the nervous systems of arthropods causing loss of coordination and paralysis, can results in a reduction of filtration rather than in directs effects on food assimilation. In support of this, Jones et al. (1991) established a relation between thoracic appendage beat and feeding rate when exposed *Daphnia catawa* to sodium dodecyl sulfate (SDS).

In the present study, the filtering rate experiment confirmed the close relation between the feeding inhibition and the small size of exposed animals. Therefore, it is possible to conclude that fenvalerate exposure resulted in a transient inhibition of the filtering rate that subsequently caused a retardation (but not an inhibition) of somatic growth - small animals on day 4 recovered on day 10 (Fig. 6). The life-table response experiment shows that the size of the animals was highly correlated with the age to first reproduction, indicating that the small animals resulted from fenvalerate exposure started to reproduce later than those in control treatments with bigger size (Fig. 7a). The delay in development caused the inhibition of reproductive output (i.e. offspring per female) (Fig. 7b) and ultimately affected the population growth rate (Table 1). However, the initial losses could be buffered as females increased the number of released offspring (Fig. 8). It is crucial to notice that the short-term exposure (24-h) of fenvalerate impaired the population growth rate at concentrations that also affected feeding responses (≥ 0.3 µg/L). Barata et al. (2002) found a similar relationship between short-term exposures to cypermethrin, closely related pyrethroid, and long-term demographic responses of the copepod *Acartia tonsa*. Feeding responses of *Daphnia ssp*. anticipated also changes in population densities when enclosed freshwater planktonic communities were contaminated with fenvalerate (Day et al, 1987).

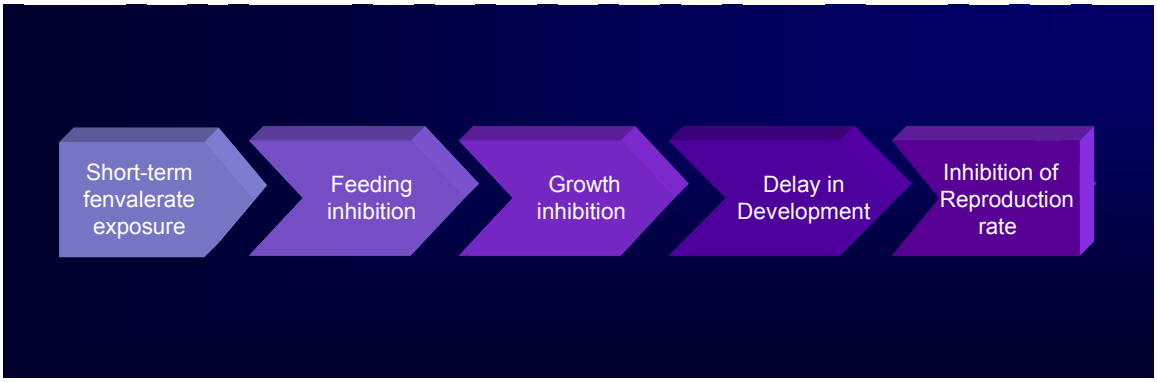


Fig. 9. Fenvalerate effects on feeding behavior that cause a transient inhibition of the population growth rate delaying age at first reproduction (i.e. delay in development).

In conclusion, the results of the three experiments performed in this study how clearly that a short-term (24h) exposure to fenvalerate can cause a transient inhibition of the filtering rate of *D. magna*, and then leading to a growth retardation delaying development with the subsequently inhibition and recovery of the population growth rate. This clearly indicates the importance of understanding feeding responses to predict the impact of stressors at high organizational levels.

SUMMARY

Pesticide registrants and regulators have been recommended a tiered-process to assess the risks of pesticides in aquatic ecosystems. The common approach within each Tier a based on conservative assumptions is to evolve towards reduction of uncertainties in risk assessment. Tier 1, a simple worst-case estimation of environmental concentration of the tested pesticide, is compared with the effect level for the most sensitive species (the hazard quotient approach). If this hazard quotient suggests a potential hazard, further tiers of risk assessment with more realistic complete exposure and toxic effects data should be incorporated into the assessment (i.e. High Tiers: 2, 3 and 4). Many questions raised and issues emerged for discussion and further development from this proposal when High Tiers are incorporated. There are various gaps of knowledge in pesticide risk assessment. Some of them were investigated in the present work and are the followings:

1. The effects of pulse or short-time exposures to pesticides.
2. The extrapolation of toxic effects from individual-level to population-level.
3. Feeding responses: link between individual and population levels.

1. The effects of pulse or short-time exposures to pesticides.

Concentrations of hydrophobic pesticides in streams decrease quickly because of adsorption processes. Thus, contamination of surface waters from hydrophobic pesticides occurs in pulses and the effects of pulsed exposures are a source of uncertainty in the pesticide risk assessment. Therefore, it is of interest to examine what are the effects of more realistic exposure scenarios, including effects that might persist after a pulse contamination, compared to those predicted from standard risk assessment scenarios (i.e. usually continuous exposure).

2. The extrapolation of toxic effects from individual-level to population-level.

Stress factors such as pesticide toxicity can affect individuals, determining birth and death rates. The population growth rate, which integrates both birth and death rates, determines the net effects of toxic effects acting on individuals. Life table response experiments (LTRE) provide a measure of effect on population growth rate under toxicant exposure. The true nature of how populations are maintained under natural conditions is not fully understood; therefore, the impact of changes to birth rates, death rates, and recruitment caused by the introduction of toxicants is

also not fully understood. Some of the sources of uncertainty for population-level analysis reside in the lack of understanding of population dynamics.

3- Feeding responses: link between individual and population levels.

Studies of populations provide information about the existence the effects of stress but do not about the causes and the mechanisms. Understanding behavioral responses of individuals to stress such as feeding responses is essential in order to understand why species differ in their susceptibility to stressors and how populations can persist in contaminated environments.

Objectives and experimental system

The aim of the present work is to contribute to the refinement of the risk assessment of pesticides. The life-table responses of *Daphnia magna* Straus exposed to continuous and pulse contamination of the pyrethroid insecticide fenvalerate were investigated under two different food levels (Fig. 1). The life table data were used to apply two population models (a fully-age classified model and a simply two-stages model). The fully-age classified model was used to generate population growth values in order to extrapolate effects on individual-level traits (i.e. survival, reproductive output, age to first reproduction) to population-level responses. The simply two-stages model was used to calculate: 1- The contributions of the individual-level traits to the inhibition of the population growth rate following a continuous and pulse exposure of fenvalerate, and 2- The sensitivity of the population growth rate to changes in individual-level traits (i.e. elasticities). In addition, the relationship between feeding and population-level responses of *D. magna* to a pulse contamination of fenvalerate was investigated (Fig. 1).

Daphnia magna was chosen because this specie plays a central role in pelagic food webs of temperate and arctic lakes and ponds, feeding on microalgae and bacteria. On the other hand, *Daphnia* are a preferred food item for fish and invertebrate predators. In addition, on algae and is currently used for ecotoxicological testing because *Daphnia magna* is easy to culture and has a short generation time. The synthetic pyrethroid Fenvalerate was chosen because its high toxicity to arthropod such *D. magna* in standard laboratory studies that justifies the implementation of more refined tiers of risk assessment (i.e. High Tiers).

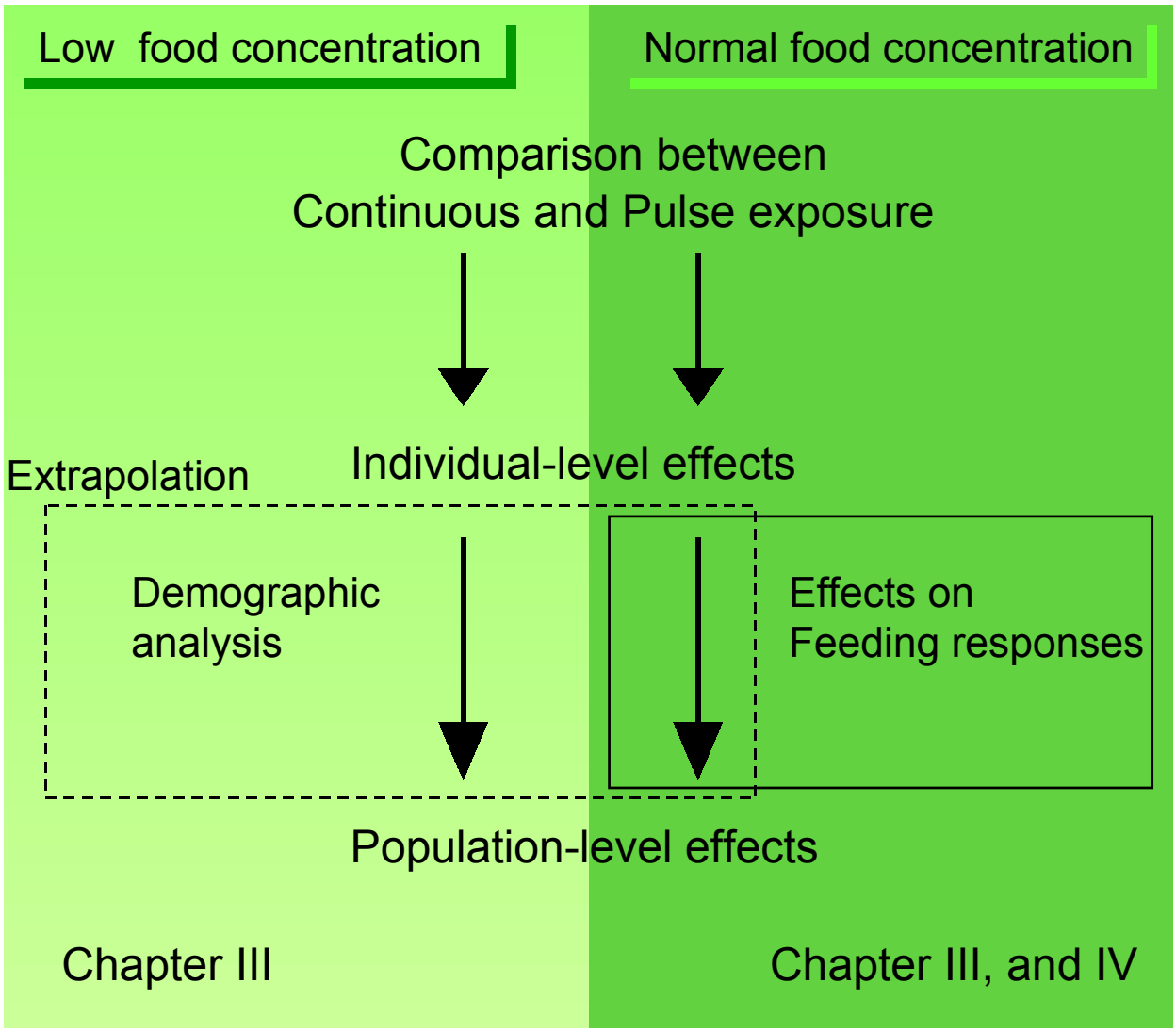


Fig.1 Schema of the dissertation, which show the organization of the investigation about the gaps of knowledge in pesticide risk assessment, which were performed under restrictive food conditions and normal food conditions (i.e. no restrictive food).

Experimental investigations

In the Chapter II, the responses of *Daphnia magna* Straus exposed to fenvalerate continuously (21d) and as a pulse (24h) were compared. This investigation is summarized in the chapter II. Survival was more severely affected in the continuous than in the pulse exposure regimes; indeed, 100% mortality occurred after exposed individuals to 1 and 3.2 µg/L of fenvalerate in the continuous and pulse exposure regimes, respectively. Age to first reproduction was delayed for both exposure regimes, and this affected both reproduction and population growth rate. After 10 days, the inhibition in reproduction and decrease in population growth rate were observed at similar concentration in both regimes, indicating that the fenvalerate toxicity at low concentrations is more related to its concentration than to the exposure duration. By contrast,

recovery from sublethal effects differed in both regimes. After pulse exposure, individuals recovered totally; under continuous exposure, the recovery was only partial. After 21 days, the reproduction was severely reduced and the population growth rate was negative in the continuous regime (0.6 µg/L). In view of the lack recovery in the continuous exposure regime, it follows that a concentration of fenvalerate sufficient to cause the extinction of a population exposed in the long term may be more than 5 times lower than that found for the pulse exposure regime.

In the Chapter III, the demographic responses of *Daphnia magna* exposed to continuous (21d) and pulse (24h) contamination were investigated. Two main hypothesis were tested: 1- The differences between responses of *D. magna* to pulse and continuous exposure (observed in Chapter II), when food is restricted; 2- The risk assessments based on individual-level traits (e.g. survival) may be overprotective in terms of population-level (i.e. population growth rate under food restriction, in particular for iteroparous species such as *D. magna.*

In the present chapter, survival responses differed between exposure regimes. Complete mortality in the continuous regime occurred at more than five times lower concentration than in the pulse regime. Therefore, the difference between exposures regimes in the fenvalerate concentrations that caused the complete mortality (i.e. 100% mortality) of daphnia individuals was bigger under food restriction than under normal food (3.3 times)(Chapter II). The sublethal responses to fenvalerate in both exposure regimes were a retardation (but not an inhibition) of somatic growth (smaller sizes of individuals) as well as a delay in the age of first reproduction and a smaller number of broods. These sublethal effects were all related since the size of the individuals on day 7 was highly correlated to the age at first reproduction, which was highly correlated with the number of broods. This was observed under both exposure regimes. The reduction of the number of broods per female produced initially a severe inhibition in the number of neonates per female. However, these initial losses were rapidly buffered as females progressed then normally with the reproduction process (i.e. increase of neonates per female over time).

The population growth rate was severely inhibited at beginning of reproduction, but this inhibition was also later on buffered with the progress the reproduction. Nevertheless, population growth rate after day 21 was highly correlated with the mortality registered at the beginning of the experiment that was more important when individuals were exposed continuously to fenvalerate. The decomposition analysis confirmed that this same endpoint (mortality observed at the beginning of the experiment or in other words juvenile survival) was the major contribution

to the inhibition of the population growth rate observed even after 21 days. In addition, the elasticity analysis showed that population growth rate was more sensitive to the changes in juvenile survival than to age at first reproduction. The relevance of elasticity to juvenile survival seems to be in accordance with the hypothesis that the evolution of increased iteropatity in *Daphnia* species responds to low reproductive success due to competition and predation.

When comparing continuous and pulse exposure under food restriction (Chapter III) and normal food (Chapter II), the differences observed between survival responses were more important when food was restricted. Nonetheless, fenvalerate under normal food condition affected age at first reproduction in a similar way than under food restriction, under both exposure regimes. However, the population growth rate in the pulse regime under food restriction did not recover in the same extent as under normal food conditions, where values after 21 days were similar to those in control treatments (Chapter II). This occurred most likely because of two reasons: 1) mortality was more severe under food restriction, and 2) under food restriction females released less broods making difficult to buffer the impact of the initial losses.

Giving support to the first hypothesis, the population growth rate on day 21 under food restriction (Chapter III) was highly correlated with the mortality with steeper slope in the continuous exposure. In addition, the decomposition analysis confirmed that mortality was the main contribution to the inhibition of the population growth rate on day 21 in both exposure regimes. It is worth to notice that these sublethal effects (i.e. developmental delay) would be more relevant in case of semelparous species, because the buffering mechanism cannot take place in these species. The elasticity analysis showed that the population growth rate in the continuous exposure was more sensitive to changes the survival juvenile than in the pulse exposure. Fenvalerate effects on survival were lessened at population-level in the pulse exposure.

In the Chapter IV, the relationship between fenvalerate-induced changes in the feeding behavior of *D. magna* (filtering species) and the subsequent population-level following 24h-pulse effects was investigated. By using ^{15}N-tracer incorporation, ^{15}N-tracer turnover over time, and filtering experiments, the feeding responses were studied and compared with results of *Daphnia* reproduction test (i.e. life table response experiment). The results of ^{15}N-tracer incorporation showed that the significant lower ^{15}N abundance [atom-%] and small size observed in individuals exposed to a 24h-puse of fenvalerate was associated with an inhibition of the processes of food uptake and assimilation.

The ^{15}N- tracer elimination (or turnover experiment) confirmed that a short-term fenvalerate exposure (24h) could affect the feeding process retarding the somatic growth. The filtering rate experiment demonstrated that the decrease in ^{15}N abundance observed in the ^{15}N-tracer experiment was likely caused by a transient inhibition of filtering rate, and confirmed the close relation between the feeding inhibition and the small size of exposed animals.

The life-table response experiment (Chapter IV) showed that the size of the animals was highly correlated with the age to first reproduction and confirmed the relationship between the growth retardation and the delay in development (i.e. increase of age at first reproduction) previously observed in Chapter III. This delay caused the inhibition of reproductive output (i.e. offspring per female) and ultimately affected the population growth rate. However, the initial losses could be buffered as females increased the number of released offspring in a similar that observed previously in Chapters II and III. It is crucial to notice that the short-term exposure (24-h) to fenvalerate impaired the population growth at concentrations that also affected feeding responses as shown in chapter IV.

The comparison of the results of these independent experiments shows clearly that a short-term (24h) exposure to fenvalerate caused a transient inhibition of *D. magna* feeding responses. This has the consequence to delay the development (i.e. age at first reproduction) through the growth retardation. As observed in the previous chapters, the delay in development caused a transient inhibition of the population growth. It is important to note that inhibition and recovery of the population growth rate and feeding responses occurred at the same concentrations ($\geq$ 0.3 µg/L). This indicates the utility of the feeding responses of individuals to understand and predict the impact of stressors at higher organizational levels.

REFERENCES

Abel P.D. and S.M. Garner. 1986. Comparisons of median survival times and median lethal exposure times for *Gammarus pulex* exposed to cadmium, permethrin and cyanide. *Water Res.* 20: 579–582.

Adelsbach, T. and R.S. Tjeerdema. 2003. Chemistry and fate of fenvalerate and esfenvalerate. *Rev. Environ. Contam. Toxicol.* 176, 137-154

Adema, D. M. 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hidrobiologia.* 59: 125-134.

Agnihotri N.P., Jain H.K., and V.T. Gajbhiye 1986. Persistence of some synthetic pyrethroid insecticides in soil, water and sediment -Part I. *J. entomol. Res.*10**:** 147-151.

Allen Y., Calow P., and D. Baird. 1995. A mechanistic model of contaminant-induced feeding inhibition in Daphnia magna. *Environ. Toxicol. Chem.* 14: 1625–1630.

American Society for Testing and Materials. 1997. Guide for conducting renewal life-cycle toxicity tests with *Daphnia magna.* E 1193-96: In *ASTM Standards on Aquatic Toxicology and Hazard Evaluation,* Vol. 11.05. Philadelphia, PA, pp 474–491.

Anderson R.L. 1982. Toxicity of fenvalerate and permethrin to several non-target aquatic invertebrates. *Environ. Entomol.*11**:** 1251-1257.

Baird, D. J., Barber I., and P. Calow. 1990. Clonal variation in general responses of Daphnia magna Straus to toxic stress. I. Chronic life-history effects. *Funct. Ecol.* 4: 399–407.

Barata C. and D.J. Baird. 2000. Determining the ecotoxicological mode of action of chemicals from measurements made on individuals: Results from instar-based tests with *Daphnia magna* Straus. *Aquat. Toxicol.* 48: 195–209.

Barata C., Baird D.J. and M. V.M.A. Soares. 2002. Demographic responses of a tropical cladoceran to cadmium: effects of food supply and density. *Ecol. Applic.* 12: 552–564.

Barry M.J., Logan D.C., Ahokas J.T., and D.A. Holdway. 1995. Effects of algal food concentration on toxicity of two agricultural pesticides to *Daphnia carinata*. *Ecotoxicol*. *Environ*. *Saf*. 32: 273-279.

Baughman, D.S., Moore, D.W. and G.I. Scott. 1989. A comparison and evaluation of field and laboratory toxicity tests with fenvalerate on an estuarine crustacean. *Environ. Toxicol. Chem.* 8:417–429.

Boersma 1995. Competition in natural populations of *Daphnia. Oecologia.* 103:309-318

Boxall A., Brown C. and K. Barrett. 2001. Higher tier laboratory aquatic testing. Cranfield for Ecochemeitry research report No. JF 4317E for DETR, 70pp.

Calow P. and R. M. Sibly. 1990. A physiological basis of population processes: ecotoxicological implications. *Funct. Ecol.* 4: 283–288.

Calow P., Sibly R.M., and V.E. Forbes. 1997. Risk assessment on the basis of simplified life-history scenarios. *Environ. Toxicol. and Chem.* 16: 1983–1989.

Caswell H. 1996. Demography meets ecotoxicology: Untangling the population level effects of toxic substances: In M.C. Newman and C.H. Jagoe, eds., Ecotoxicology: A Hierarchical Treatment. CRC, Boca Raton, FL, USA, pp. 255–317.

Caswell H. 2000 Matrix population models: construction, analysis, and interpretation, second edition. Sunderland, MA. Sinauer.

Clark J.M. and F. Matsumura. 1982. Two different types of inhibitory effects of pyrethroids on nerve Ca^- and Ca^+Mg ATPase in the squid, *Loligo paelei*: *Pestic Biochem Physiol.* 4:232–238.

Clark J.M. and G.M. Brooks. 1989. Neurotoxicology of pyrethroids: Single or multiple mechanisms of action. *Environ Toxicol Chem* 8:361–372.

Clark J.R., Goodman L.R., Borthwick P.W., Patrick J.M.J., Cripe G.M., Moody P.M., Moore J.C. and E.M. Lores. 1989. Toxicity of pyrethroids to marine invertebrates and fish: A literature review and test results with sediment-sorbed chemicals. *Environ. Toxicol. Chem.* 8:393–401.

Cold A. and V.E. Forbes. 2004. Consequences of a short pulse of pesticide exposure for survival and reproduction of *Gammarus pulex*. *Aquat. Toxicol*. 67: 287-299.

Daniels R.E. and J.D. Allan. 1981. Life table evaluation of chronic exposure to a pesticide. *Can. J. Fish. Aquat. Sci.* 38: 485-495.

Day K. and N.K. Kaushik. 1987a. An assessment of the chronic toxicity of the synthetic pyrethroid, Fenvalerate, to *Daphnia galeata mendotae*, using life tables. *Environ. Pollut.* 44: 13-26.

Day K and N.K. Kaushik 1987b. Short-term exposure of zooplankton to the synthetic pyrethroid fenvalerate, and its effects on rates of filtration and assimilation of the alga, *Chlamydomonas reinhardii. Arch. Environ. Contam. Toxicol.* 16:423–432.

Day K. and N.K. Kaushik. 1987c. The adsorption of fenvalerate to laboratory glassware and the alga *Clamydonas reinhardii*, and its effects on uptake of the pesticide by Daphnia galeata mendotae. *Aquat. Toxicol*. 10: 131–142.

Day, K, Kaushik N.K. and K.R. Solomon. 1987. Impact of fenvalerate on enclosed freshwater planktonic communities and on *in situ* rates of filtration of zooplankton. *Can. J. Fish Aquat. Sci.* 44:1714–1728.

Day K. 1991. Effects of dissolved organic carbon on accumulation and acute toxicity of fenvalerate, delthamethrin and cyhalothrin to Daphnia magna (Straus). *Environ. Toxicol. Chem. 10*: 91-101.

deKroon, H., Plaisier A., Van Groenendael J. and H. Caswell. 1986. Elasticity: the relative contribution of demographic parameters to population growth rate. *Ecology* 67:1427-1431.

Ebert D. 1994. A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. *Oikos*. 69:309-317.

Ebert, D., Yampolsky, L.Y. & Stearns, S.C. 1993. Genetics of life history traits in *Daphnia magna*: I. Heritabilities at two food levels. Heredity, 70:335-343.

Ecology and Contaminants Project, U.S. Geological Survey http://wwwrcamnl.wr.usgs.gov/tracel/

Environment Canada. 1997. Environmental Assessments of Priority Substances under the Canadian Environmental Protection Act. Guidance Manual Version 1.0. EPS/2/CC/3E. Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada. ISBN 0-662-25403-1.

EPA 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F

EU Council Directive. 1991. Concerning placing of plant protection products on the market. 91/414/EEC of July 1991.Official Journal of the European Communities L 230, 19.08.91.

European Union. 1991. Council Directive 91/414/EEC Concerning the Placing of Plant Protection Products on the Market. Official Journal of the European Communities. July 15, 1991.

Farmer, D., Hill I.R. and S.J. Maund. 1995. A comparison of the fate and effects of two pyrethroid insecticides (lambdacyhalothrin and cypermethrin) in pond mesocosms. *Ecotoxicol.* 4: 219-244.

Forbes V.E. and Calow P. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environ. Toxicol. Chem.* 18: 1544-1556.

Forbes V.E. and P. Calow. 2002. Population growth rate as a basis for ecological risk assessment of toxic chemicals. *Phil. Trans. R. Soc. Lond. B.* 357: 1299–1306.

Gliwicz, Z. M. 1990. Food thresholds and body size in cladocerans. Nature. 343:638–640.

Grandy N. 1991. Successful international cooperation: the *Daphnia magna* Reproduction test saga. *Environ. Toxicol. Chem.* 10: 1991-192.

Grimme, L.H. and N.K. Boardman. 1972. Photochemical activities of a particle fraction P1 obtained from the green alga *Chlorella fusca*. *Biochem Biophys Res Commun.* 49: 1617-1620.

Gurney, W.S.C., McCauley E., Nisbet R.M., and W.W. Murdoch. 1990. The physiological ecology of *Daphnia*: a dynamic model of growth and reproduction. *Ecology* 71:703-715

Hall L.W. and R.D. Anderson. 1995. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. *Crit Rev Toxicol* 25:281–346.

Hansen F.T., Forbes V.E. and T.L. Forbes. 1999 The effects of chronic exposure to 4-n-nonylphenol on life-history traits and population dynamics of the polychaete *Capitella* sp. I. *Ecol. Applic.* 9: 482–495.

Haya K. 1989. Toxicity of pyrethroid insecticides to fish. *Environ. Toxicol. Chem.* 8:391–391.

Hanazato T. 1998. Growth analysis of *Daphnia* early juvenile stages as an alternative method to test the chronic effect of chemicals. *Chemosphere* 36:1903–1909.

Holdway D.A., Barry M.J., Logan D.C., Robertson, D., Young V. and J.T. Ahokas. 1994 Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). *Aquat. Toxicol.* 28(3-4): 169-187.

Hosmer A.J., Warren L.W. and T.J. Ward. 1998. Chronic toxicity of pulse-dosed fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. *Environ. Toxicol. Chem*. 17: 1860-1866.

Jarvinen A.W., D.K. Tanner and E.R. Kline. 1988. Toxicity of chlorpyrifos, endrin, or fenvalerate to fathead minnows following episodic or continuous exposure. *Ecotoxicol. Environ. Saf*. 15: 78-95.

Kammenga J.E., Busschers M., van Straalen N.M., Jepson P.C. and J. Bakker 1996. Stress-induced fitness reduction is not determined by the most sensitive life-cycle trait. *Funct. Ecol.* 10: 106-111.

Kammenga J.E. Van Gestel C.A.M. and E. Hornung. 2001. Switching life-history sensitivities to stress in soil invertebrates. *Ecol. Applic.* 11: 226–238.

Klok, C., and A. M. de Roos. 1996. Population level consequences of toxicological influences on individual growth and reproduction in *Lumbricus rubellus* (*Lumbricidae, Oligochaeta*). *Ecotoxicol. Environ. Saf.* 33: 118–127.

Kooijman S.A.L.M. and J.A.J. Metz. 1984. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. *Ecotoxicology and Environmental Safety* 8: 254-274.

Kooijman, S. A. L. M. 1995. The stoichiometry of animal energetics. Journal of Theoretical Biology 177:139–149.

Kreuger J. 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. *Sci. Tot. Environ.* 216: 227–251.

Lampert W., and I. Trubetskova. 1996. Juvenile growth rate as a measure of fitness in *Daphnia. Funct. Ecol.* 10: 631–635.

Levi, H. W. 1959. *Daphnia. Ecology.* 40: 529–529.

Levin L., Caswell H., Bridges T., DiBacco C., Cabrera D. and G. Plaia. 1996. Demographic responses of estuarine polychaetes to pollutants: life table response experiments. *Ecol. Applic.* 6: 1295–1313.

Liess, M. and R. Schulz. 1996. Chronic effects of short-term contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus. Hydrobiologia.* 324:99–106.

Liess, M., Schulz, R., Liess, M.H.D., Rother, B. and R. Kreuzig. 1999. Determination of insecticide contamination in agricultural headwater streams. *Water Res.* 33:239–247.

Linden E., Bengtsson B.E., Svanberg O. and G. Sundstrom. 1979 The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak *(Alburnus alburnus)* and the harpacticoid *Nitocra spinipes. Chemosphere.* 11: 843-851.

Macagno E.R., LoPresti V., and C. Levinthal, 1973. Structure and development of neuronal connections in isogenic organisms: Variations and similarities in the optic system of *Daphnia magna*. *Proc. Nat. Acad. Sci. U. S.* 70: 57-61.

Maltby L. 1999. Studying stress: the importance of organism-level responses. *Ecol. Applic.* 9: 431–440.

Maund S., Hamer J.M., Warrinton J. And T.J. Kedwards. 1998. Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: considerations for higher-tier aquatic risk assessment. *Pestic. Sci.* 54: 408- 417.

Maund, S.J., Travis, K.Z., Hendley, P., Giddings, J.M. and K. Solomon. 2001. Probabilistic risk assessment of cotton pyrethroids: V. Combining landscape-level exposures and ecotoxicological effects data to characterize risks. *Environ. Toxicol. Chem.* 20:687–692.

Mayer F.L. Jr. and M.R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals, Washington, DC, US Department of the Interior, Fish and Wildlife Service, pp. 235-236.

McCahon, C.P. and Pascoe, D., 1988. Culture techniques for three freshwater macroinvertebrate species and their use in toxicity tests. *Chemosphere.* 17: 2471–2480.

McCauley E., Murdoch W.W., Nisbet R.M. and W. S. C. Gurney. 1990. The physiological ecology of *Daphnia*: development of a model of growth and reproduction. *Ecology*. 71:703–715.

McWilliam R.A. and Baird D.J. 2002. Postexposure feeding depression: a new toxicity endpoint for use in laboratory studies with daphnia magna. *Environ. Toxicol. Chem.* 21: 1198–1205.

Meyer, J.S., Ingersoll, C.G., L.L. McDonald and M.S. Boyce. 1986. Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecolog*y. 67: 1156-1166.

Naddy, R.B., Johnson, K.A. and S.J. Klaine. 2000. Response of *Daphnia magna* to pulsed exposures of chlorpyrifos. *Environ. Toxicol. Chem.* 19:423–431.

Ohno N., Fujimoto K., Okuno Y., Mizutani T., Hirano M., Itaya N., Honda T. and H. Yoshioka. 1976. 2-Arylalkanoates, a new group of synthetic pyrethroid esters not containing cyclopropane-carboxylates. *Pestic. Sci.,* 7**:** 241-246.

OECD. 1998. *Daphnia magna* Reproduction Test. Guidelines for Testing of Chemicals No. 211. Organisation for Economic Co-operation and Development. Paris, France.

Potvin, C. and D.A. Roff. 1993. Distribution-free and robust statistical methods - viable alternatives to parametric statistics. *Ecology.* 74: 1617-1628.

Ratte, H.T. 1996. Statistical implications of end-point selection and inspection interval in the daphnia reproduction test: a simulation study. *Environ. Toxicol. and Chem.* 15: 1831–1843.

Reinert, K.H., Giddings, J.M. and L. Judd. 2002. Effects analysis of time-varying or repeated exposures in aquatic ecological risk assessment of agrochemicals. *Environ. Toxicol. Chem.* 21:1944–1992.

Rose R.M., Warne M.St.J. and R.P. Lim. 2002. Food concentration affects the life history response of *ceriodaphnia cf. dubiato* to chemicals with different mechanisms of action. *Ecotoxicol. Environ. Saf.* 51: 106-114.

Rowe, C.L. and Hebert, P.D.N. 1999. Cladoceran Web Site. University of Guelph. www.cladocera.uoguelph.ca

Santojanni A., Gorbi G. and F. Sartore. 1998. Prediction of fecundity in chronic toxicity tests on *Daphnia magna*. *Wat. Res.* 32: 3146–3156.

Schulz, R. and M. Liess. 1999. Validity and ecological relevance of an active in situ bioassay using *Gammarus pulex* and *Limnephilus lunatus. Environ. Toxicol. Chem.* 18:2243–2250.

Schulz, R. and M. Liess. 2000. Toxicity of fenvalerate to caddisfly larvae: Chronic effects of 1- vs. 10-h pulse exposure with constant doses. *Chemosphere.* 41:1511–1517.

Schulz R. and M. Liess. 2001. Toxicity of the aquatic-phase and suspended particle-associated fenvalerate: chronic effects after pulse exposure-dose exposure *Limnephilus lunatus* (Thricoptera). *Environ. Toxicol. Chem.* 20:185-190.

Schwartz, S.S.1984. Life history Strategies in Daphnia: a review and predictions. *Oikos*. 42: 114-122.

Scott, G.I. et al. 1999. Assessment of risk reduction strategies for the management of agricultural non-point source pesticide runoff in estuarine ecosystem. *Toxicol. Ind. Health.* 15:200–213.

Sibly R.M. 1999. Efficient experimental designs for studying stress and population density in animal populations. *Ecol. Applic*. 9: 496–503.

Sibly R.M., Hansen F.T. and V.E. Forbes. 2000. Confidence intervals for population growth rate of organisms with two-stage life histories. *Oikos* 88: 335–340.

Siegfried BD. 1993. Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. *Environ. Toxicol. Chem.* 12:1683–1689.

60 Ways Farmers Can Protect Surface Water. This Land (online publication). University of Illinois Extension http://www.thisland.uiuc.edu/60ways/60ways_54.html

Society of Environmental Toxicology and Chemistry (SETAC). 1994. Aquatic Dialogue Group: Pesticide Risk Assessment and Mitigation. Baker J.L., Barefoot A.C., Beasley L.A., Burns P.P., Caulkins J.E. and R.L. Clark.

Solomon K.R., Giddings J.M. and S.J. Maund. 2001. Probabilistic risk assessment of cotton pyrethroids in aquatic ecosystems: I. Distributional analysis of laboratory aquatic toxicity data. *Environ. Toxicol. Chem.* 20: 652–659.

Stark J.D. and J.A.O Banken. 1999. Importance of population structure at the time of toxicant exposure. *Ecotoxicol. Environ. Saf.* 42: 282-287.

Stark, J.D., and W.K. Walthall. 2003. Agricultural adjuvants: acute mortality and effects on population growth rate of *Daphnia pulex* after chronic exposure. *Environ. Toxicol. Chem.* 22:3056–3061.

Suter G.W. II 1993. Ecological Risk Assessment. Lewis Publishers, Chelsea, Michigan. 538 pp.

Trubetskova, I. and Lampert W. 2002. The juvenile growth rate of D*aphnia*: a short-term alternative to measuring the per capita rate of increase in ecotoxicology? *Arch. Environ. Contam. Toxicol.* 42, 193–198.

U.S. EPA (Environmental Protection Agency). 1998. Guidelines for ecological risk assessment. EPA/630/OR-95/002F. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

Urban D. J., and N. J. Cook. 1986. *Hazard Evaluation Division Standard Evaluation Procedure: Ecological Risk Assessment*. EPA 540/9-85-001. United States Environmental Protection Agency, Washington, D.C. issued June 1986. (NTIS PB86-247657).

van Groenendael J., DeKroon H., Kalisz S. and S. Tuljapurkar. 1994. Loop analysis: Evaluating life history pathways in population projection matrices. *Ecology* 75:2410-2415.

van Tienderen. P.H. 1995. Life cycle trade-offs in matrix population models. *Ecology* 76:2482-2489.

Walthall W.K. and J.D. Stark. 1997. A comparison of acute mortality and population growth rate as endpoints of toxicological effect. *Ecotox. Environ. Safety* 37: 45-57.

Walthall W.K. and J.D. Stark. 1997. Comparison of two population-level ecotoxicological endpoints: the intrinsic (rm) and instantaneous (ri) rates of increase. *Environ. Toxicol. Chem.* 16: 1068-1073.

Woin, P. 1998. Short- and long-term effects of the pyrethroid insecticide fenvalerate on an invertebrate pond community. *Ecotoxicol. Environ. Saf.* 41:137–156.